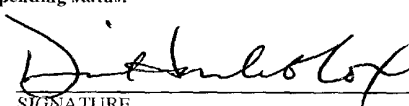


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FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER PF-0589 USN
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. 09/786133 TO BE ASSIGNED
INTERNATIONAL APPLICATION NO. PCT/US99/20468	INTERNATIONAL FILING DATE 02 September 1999	PRIORITY DATE CLAIMED 02 September 1998
TITLE OF INVENTION HUMAN MEMBRANE CHANNEL PROTEINS		
APPLICANT(S) FOR DO/EO/US INCYTE PHARMACEUTICALS, INC.; AU-YOUNG, Janice; BANDMAN, Olga; TANG, Y. Tom; REDDY, Roopa; HILLMAN, Jennifer L.; YUE, Henry; LAL, Preeti; CORLEY, Neil.; GUEGLER, Karl J.; GORGONE, Gina, BAUGHN, Mariah R.; AZIMZAI, Yalda		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.</p> <ol style="list-style-type: none"><li><input checked="" type="checkbox"/> This is the <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li><li><input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li><li><input type="checkbox"/> This is an express request to promptly begin national examination procedures (35 U.S.C. 371 (f)).</li><li><input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).</li><li><input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))<ol style="list-style-type: none"><li><input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li><li><input type="checkbox"/> has been communicated by the International Bureau.</li><li><input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li></ol></li><li><input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</li><li><input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))<ol style="list-style-type: none"><li><input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li><li><input type="checkbox"/> have been communicated by the International Bureau.</li><li><input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li><li><input checked="" type="checkbox"/> have not been made and will not be made.</li></ol></li><li><input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li><li><input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li><li><input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li></ol>		
Items 11 to 16 below concern document(s) or information included:		
<ol style="list-style-type: none"><li><input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li><li><input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.27 and 3.31 is included.</li><li><input type="checkbox"/> A <b>FIRST</b> preliminary amendment. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li><li><input type="checkbox"/> A substitute specification</li><li><input type="checkbox"/> A change of power of attorney and/or address letter</li><li><input checked="" type="checkbox"/> Other items or information.</li></ol> <p>1) Transmittal Letter (2 pp, in duplicate) 2) Return Postcard 3) Express Mail Label No.: <u>EL 856 112 747 US</u></p>		

U.S. APPLICATION NO. (OFFICE USE) <b>09/786133</b> TO BE ASSIGNED		INTERNATIONAL APPLICATION NO. PCT/US99/20468		ATTORNEY'S DOCKET NUMBER PF-0589 USN	
17 <input type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the FPO or JPO.....\$1000.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO...\$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO...\$710.00 <input checked="" type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... <b>\$690.00</b> <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....\$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$690.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	20 =	0	X \$ 18.00	\$	
Independent Claims	2 =	0	X \$ 80.00	\$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$690.00	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$	
SUBTOTAL =				\$690.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$690.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by the appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$690.00	
				Amount to be Refunded:	\$
				Charged	\$
a. <input type="checkbox"/> A check in the amount of \$_____ to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. <u>09-0108</u> in the amount of \$ <u>690.00</u> to cover the above fees c. <input type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>09-0108</u> . A duplicate copy of this sheet is enclosed.					
<b>NOTE:</b> Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SFND ALL CORRESPONDENCE TO:  INCYTE GENOMICS, INC. 3160 Porter Drive Palo Alto, CA 94304					
 SIGNATURE					
NAME: Diana Hamlet-Cox					
REGISTRATION NUMBER: 33,302					
DATE: <u>27</u> February 2001					

## HUMAN MEMBRANE CHANNEL PROTEINS

### TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of human membrane  
5 channel proteins and to the use of these sequences in the diagnosis, treatment, and prevention of  
cell proliferative, immune/inflammatory, transport/secretory, osmoregulatory, muscular,  
cardiovascular, and neurological disorders.

### BACKGROUND OF THE INVENTION

10 Channel proteins facilitate the transport of hydrophilic molecules across membranes by  
forming aqueous pores that can perforate a lipid bilayer. Many channels consist of protein  
complexes formed by the assembly of multiple subunits, at least one of which is an integral  
membrane protein that contributes to formation of the pore. In some cases, the pore is constructed  
to selectively allow passage of only one or a few molecular species. Distinct types of membrane  
15 channels that differ greatly in their distribution and selectivity include: (1) aquaporins, which  
transport water; (2) protein-conducting channels, which transport proteins across the endoplasmic  
reticulum membrane; (3) gap junctions, which facilitate diffusion of ions and small organic  
molecules between neighboring cells; and (4) ion channels, which regulate ion flux through  
various membranes.

#### 20 Aquaporins

Aquaporins (AQP) are channels that transport water and, in some cases, nonionic small  
solutes such as urea and glycerol. Water movement is important for a number of physiological  
processes including renal fluid filtration, aqueous humor generation in the eye, cerebrospinal fluid  
production in the brain, and appropriate hydration of the lung. A variety of aquaporins have been  
25 found in higher animals, plants and microorganisms. The mammalian aquaporins appear to have  
selective expression in particular tissues, with AQP0 localized to lens epithelium; AQP1 localized  
to many tissues including red blood cells, kidney, eye, lung, choroid plexus, bile duct, and  
vascular epithelium; AQP2 localized to the apical membrane of kidney collecting duct cells; AQP3  
localized to kidney, colon, trachea, urinary bladder, skin, and sclera of eye; AQP4 localized to  
30 kidney, colon, trachea, stomach, skeletal muscle, spinal cord, brain, and retina; AQP5 localized to  
the apical membranes of exocrine tissues; AQP6 localized to kidney; and AQP7 localized to testis  
(King, L.S. and P. Agre (1996) Annu. Rev. Physiol. 58:619-648; Ishibashi, K. et al. (1997) J. Biol.  
Chem. 272:20782-20786). AQP9 is expressed in peripheral leukocytes, less abundantly in liver,  
even less in lung and spleen, and not at all in thymus tissue (Ishibashi, K. et al. (1998) Biochem.

Biophys. Res. Commun. 244:268-274).

Aquaporins are members of the major intrinsic protein (MIP) family of membrane transporters. MIP family proteins are composed of four subunits, each of which may span the membrane six times, and have their N-and C-termini facing the cell cytoplasm. Proteins from bacteria, yeast, plants, and animals have been shown to be members of the MIP family (Reizer, J. et al. (1993) Crit. Rev. Biochem. 28:235-257). Aquaporin subunits are integral membrane proteins with six transmembrane regions and two conserved Asn-Pro-Ala (NPA) boxes (which are sometimes found as Asn-Pro-Ser) found in loop regions between the transmembrane regions (King, supra; Ishibashi, (1997) supra). The study of aquaporins may have relevance to understanding edema formation and fluid balance in both normal physiological and disease states (King, supra). Mutations in AQP2 cause autosomal recessive nephrogenic diabetes insipidus (Online Mendelian Inheritance in Man (OMIM) \*107777 Aquaporin 2; AQP2). Reduced AQP4 expression in skeletal muscle may be associated with Duchenne muscular dystrophy (Frigeri, A. et al. (1998) J. Clin. Invest. 102:695-703). Mutations in AQP0 cause autosomal dominant cataracts in mice (OMIM \*154050 Major Intrinsic Protein of Lens Fiber; MIP).

#### Protein-Conducting Channels

Secreted and integral membrane proteins are transported from the cytoplasm to the endoplasmic reticulum (ER) through protein-conducting channels in the ER membrane. The channel is used for both co- and post-translational translocation. In the co-translational process, transport is initiated by the action of a cytoplasmic signal recognition particle (SRP) which recognizes a signal sequence on a growing, nascent polypeptide and binds the polypeptide and its ribosome complex to the ER membrane through an SRP receptor located on the membrane. The ribosome complex, together with the attached polypeptide, becomes membrane bound. As the nascent chain emerges from the ribosome, it is fed into the channel and across the ER membrane. The post-translational process also requires a signal sequence on the protein to be translocated, but does not require an SRP. The protein enters the channel and is driven across the ER membrane by the hydrolysis of adenosine triphosphate (ATP) by BiP, an ATPase and molecular chaperone in the ER lumen.

The protein-conducting channel, termed the Sec61p complex, is composed of multiple, probably two, heterotrimers of three membrane proteins, the alpha, beta, and gamma subunits of Sec61p. The Sec61p complex forms a ring structure visible by electron microscopy (EM). EM and quenching experiments indicate a channel diameter of 20 to 60 Å. Association of the Sec61p complex with the ribosome and with the proteins Sec62p, Sec63p, Sec71p, Sec72p, BiP, and TRAM (translocating chain-associating membrane protein) is required for some of the channel's

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functions. The Sec61p alpha subunit contains ten membrane-spanning segments and has been found to line the path of the translocating polypeptide chain from one side of the membrane to the other. The sequences of dog and rat Sec61p alpha genes have been determined. Homologs of the mammalian Sec61p alpha are found in the yeast Saccharomyces cerevisiae (Sec61p) and in  
5 bacteria (SecYp). (See Görlich, D. et al. (1992) Cell 71:489-503; Matlack, K.E.S. et al. (1998) Cell 92:381-390.)

Defects in protein trafficking to organelles or to the cell surface are involved in numerous human diseases and disorders including cystic fibrosis, glucose-galactose malabsorption syndrome, hypercholesterolemia, diabetes mellitus, diabetes insipidus, hyper- and hypoglycemia,  
10 Grave's disease, goiter, Cushing's disease, and Addison's disease. Cancer cells secrete excessive amounts of hormones or other biologically active peptides.

#### Gap Junctions

Gap junctions (also called connexons) are channels that function chemically and electrically to couple the cytoplasm of neighboring cells in many tissues. Gap junctions function  
15 as electrical synapses for intercellular propagation of action potentials in excitable tissues. In nonexcitable tissues, gap junctions have roles in tissue homeostasis, coordinated physiological response, metabolic cooperation, growth control, and the regulation of development and differentiation. Gap junctions help to synchronize heart and smooth muscle contraction, speed neural transmission, and propagate extracellular signals. Gap junctions can open and close in  
20 response to particular stimuli (e.g., pH,  $Ca^{+2}$ , and cAMP). The effective pore size of a gap junction is approximately 1.5 nm, which enables small molecules (e.g., those under 1000 daltons) to diffuse freely through the pore. Transported molecules include ions, small metabolites, and second messengers (e.g.,  $Ca^{+2}$  and cAMP).

Each connexon is composed of six identical subunits called connexins. At least thirteen  
25 distinct connexin proteins exist, with each having similar structures but differing tissue distributions. Structurally, the connexins are integral membrane proteins with four putative membrane spanning regions and N- and C-termini oriented towards the cell cytoplasm. Conserved regions include the membrane spanning regions and two extracellular loops. The variable regions, which are two cytoplasmic loops and the C-terminal region, may be responsible for the regulation  
30 of different connexins. (See Hennemann, H. et al. (1992) J. Biol. Chem. 267:17225-17233; PRINTS PR00206 connexin signature.)

Connexins have many disease associations. Female mice lacking connexin 37 (Cx37) are infertile due to the absence of the oocyte-granulosa cell signaling pathway. Mice lacking Cx43 die shortly after birth and show cardiac defects reminiscent of some forms of stenosis of the

pulmonary artery in humans. Mutations in Cx32 are associated with the X-linked form of Charcot-Marie-Tooth disease, a motor and sensory neuropathy of the peripheral nervous system. Cx26 is expressed in the placenta, and Cx26-deficient mice show decreased transplacental transport of a glucose analog from the maternal to the fetal circulation. In humans, Cx26 has been identified as the first susceptibility gene for non-syndromic sensorineural autosomal deafness. Cx46 is expressed in lens fiber cells, and Cx46-deficient mice develop early-onset cataracts that resemble human nuclear cataracts. (See Nicholson, S.M. and R. Bruzzone (1997) Curr. Biol. 7:R340-R344.)

#### Ion Channels

The electrical potential of a cell is generated and maintained by controlling the movement of ions across the plasma membrane. The movement of ions requires ion channels, which form an ion-selective pore within the membrane. There are two basic types of ion channel: ion transporters and gated ion channels. Ion transporters utilize the energy obtained from ATP hydrolysis to actively transport an ion against the ion's concentration gradient. Gated ion channels allow passive flow of an ion down the ion's electrochemical gradient under restricted conditions. Together, these types of ion channels generate, maintain, and utilize an electrochemical gradient that is used in 1) electrical impulse conduction down the axon of a nerve cell, 2) transport of molecules into cells against concentration gradients, 3) initiation of muscle contraction, and 4) endocrine cell secretion.

Ion channels share common structural and mechanistic themes. The channel consists of four or five subunits or protein monomers that are arranged like a barrel in the plasma membrane. Each subunit typically consists of six potential transmembrane segments (S1, S2, S3, S4, S5, and S6). The center of the barrel forms a pore lined by  $\alpha$ -helices or  $\beta$ -strands. The side chains of the amino acid residues comprising the  $\alpha$ -helices or  $\beta$ -strands establish the charge (cation or anion) selectivity of the channel. The degree of selectivity, or what specific ions are allowed to pass through the channel, depends on the diameter of the narrowest part of the pore.

#### Ion Transporters

Ion transporters generate and maintain the resting electrical potential of a cell. Utilizing the energy derived from ATP hydrolysis, they transport ions against the ion's concentration gradient. These transmembrane ATPases are divided into three families. The vacuolar (V) class of ion transporters includes  $H^+$  pumps on intracellular organelles, such as lysosomes and Golgi. V-class ion transporters are responsible for generating the low pH within the lumen of these organelles that is required for function. The coupling factor (F) class consists of  $H^+$  pumps in the mitochondria. F-class ion transporters utilize a proton gradient to generate ATP from ADP and

inorganic phosphate ( $P_i$ ). The phosphorylated (P) class ion transporters, including  $Na^+-K^+$  ATPase,  $Ca^{+2}$ -ATPase, and  $H^+$ -ATPase, are activated by a phosphorylation event. P-class ion transporters are responsible for maintaining resting potential distributions such that cytosolic concentrations of  $Na^+$  and  $Ca^{+2}$  are low and cytosolic concentration of  $K^+$  is high. The resting potential of the cell is utilized in many processes involving carrier proteins and gated ion channels. Carrier proteins utilize the resting potential to transport molecules into and out of the cell. Amino acid and glucose transport into many cells is linked to sodium ion co-transport (symport) so that the movement of  $Na^+$  down an electrochemical gradient drives transport of the other molecule up a concentration gradient. Similarly, cardiac muscle links transfer of  $Ca^{+2}$  out of the cell with transport of  $Na^+$  into the cell (antiport).

#### Gated Ion Channels

Gated ion channels control ion flow by regulating the opening and closing of pores. The ability to control ion flux through various gating mechanisms allows ion channels to mediate such diverse signaling and homeostatic functions as neuronal and endocrine signaling, muscle contraction, fertilization, and regulation of ion and pH balance. Gated ion channels are categorized according to the manner of regulating the gating function. Mechanically-gated channels open their pores in response to mechanical stress; voltage-gated channels (e.g.,  $Na^+$ ,  $K^+$ ,  $Ca^{+2}$ , and  $Cl^-$  channels) open their pores in response to changes in membrane potential; and ligand-gated channels (e.g., acetylcholine-, serotonin-, and glutamate- gated cation channels, and GABA- and glycine- gated chloride channels) open their pores in the presence of a specific ion, nucleotide, or neurotransmitter. The gating properties of a particular ion channel ( i.e., its threshold for and duration of opening and closing) are sometimes modulated by association with auxiliary channel proteins and/or post translational modifications, such as phosphorylation. The pore forming subunits of voltage-gated and transmitter-gated cation channels form two distinct superfamilies of conserved multipass membrane proteins.

Voltage-gated  $Na^+$  and  $K^+$  channels are necessary for the function of electrically excitable cells such as nerve, endocrine, and muscle cells. Action potentials, which lead to neurotransmitter release and muscle contraction, arise from large, transient changes in the permeability of the membrane to  $Na^+$  and  $K^+$  ions. Depolarization of the membrane beyond the threshold level opens voltage-gated  $Na^+$  channels. Sodium ions flow into the cell, further depolarizing the membrane and opening more voltage-gated  $Na^+$  channels, thus propagating the depolarization down the length of the cell. Depolarization also opens voltage-gated  $K^+$  channels. Consequently, potassium ions flow outward, leading to repolarization of the membrane. Voltage-gated channels utilize charged residues in the fourth transmembrane segment (S4) to sense voltage change. The open

state lasts only about 1 millisecond, at which time the channel spontaneously converts into an inactive state that cannot be opened irrespective of the membrane potential. Inactivation is mediated by the channel's N-terminus, which acts as a plug that closes the pore. The transition from an inactive to a closed state requires a return to resting potential.

5 Na<sup>+</sup> channels isolated from rat brain tissue are heterotrimeric complexes composed of a 260 kDa pore-forming  $\alpha$  subunit that associates with two smaller auxiliary subunits,  $\beta$ 1 and  $\beta$ 2. The  $\beta$ 2 subunit is an integral membrane glycoprotein that contains an extracellular Ig domain, and its association with  $\alpha$  and  $\beta$ 1 subunits correlates with increased function of the channel, a change in the channel's gating properties, as well as an increase in whole cell capacitance (Isom, L.L. et al. (1995) Cell 83:433-442).

10 K<sup>+</sup> channels are located in all cell types, and may be regulated by voltage, ATP concentration, or second messengers such as Ca<sup>++</sup> and cAMP. In non-excitabile tissue, K<sup>+</sup> channels are involved in protein synthesis, control of endocrine secretions, and the maintenance of osmotic equilibrium across membranes. In neurons and other excitable cells, in addition to regulating action potentials and repolarizing membranes, K<sup>+</sup> channels are responsible for setting resting membrane potential. The cytosol contains non-diffusible anions and, to balance this net negative charge, the cell contains a Na<sup>+</sup>-K<sup>+</sup> pump and ion channels that provide the redistribution of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>. The pump actively transports Na<sup>+</sup> out of the cell and K<sup>+</sup> into the cell in a 3:2 ratio. Ion channels in the plasma membrane allow K<sup>+</sup> and Cl<sup>-</sup> to flow by passive diffusion. Because of the high negative charge within the cytosol, Cl<sup>-</sup> flows out of the cell. The flow of K<sup>+</sup> is balanced by an electromotive force pulling K<sup>+</sup> into the cell, and a K<sup>+</sup> concentration gradient pushing K<sup>+</sup> out of the cell. Thus, the resting membrane potential is primarily regulated by K<sup>+</sup> flow (Salkoff, L. and T. Jegla (1995) Neuron 15:489-492).

25 K<sup>+</sup> pore-forming subunits generally have six transmembrane-spanning domains with a short region between the fifth and sixth transmembrane regions that senses membrane potential; and the amino and carboxy termini are located intracellularly. In mammalian heart, the duration of ventricular action potential is controlled by a K<sup>+</sup> current. Thus, the K<sup>+</sup> channel is central to the control of heart rate and rhythm. K<sup>+</sup> channel dysfunctions are associated with a number of renal diseases including hypertension, hypokalemia, and the associated Bartter's syndrome and Getelman's syndrome, as well as neurological disorders including epilepsy. K<sup>+</sup> channels have been implicated in Alzheimer's disease by observations that a significant component of senile plaques, beta amyloid or A beta, also blocks voltage-gated potassium channels in hippocampal neurons. (See Antes, L.M. et al. (1998) Seminar Nephrol. 18:31-45; Stoffel, M. and L.Y. Jan (1998) Nat. Genet. 18:6-8; Madeja, M. et al. (1997) Eur. J. Neurosci. 9:390-395; Good, T.A. et al.



(1996) Biophys. J. 70:296-304.)

Voltage-gated  $\text{Ca}^{+2}$  channels are involved in presynaptic neurotransmitter release, and heart and skeletal muscle contraction. The voltage-gated  $\text{Ca}^{+2}$  channels from skeletal muscle (L-type) and brain (N-type) have been purified and, though their functions differ dramatically, they have similar subunit compositions. The channels are composed of three subunits. The  $\alpha_1$  subunit forms the membrane pore and voltage sensor, while the  $\alpha_2\delta$  and  $\beta$  subunits modulate the voltage-dependence, gating properties, and the current amplitude of the channel. These subunits are encoded by at least six  $\alpha_1$ , one  $\alpha_2\delta$ , and four  $\beta$  genes. A fourth subunit,  $\gamma$ , has been identified in skeletal muscle. (See Walker, D. et al. (1998) J. Biol. Chem. 273:2361-2367; Jay, S.D. et al. (1990) Science 248:490-492.)

Chloride channels are necessary in endocrine secretion and in regulation of cytosolic and organelle pH. In secretory epithelial cells,  $\text{Cl}^-$  enters the cell across a basolateral membrane through an  $\text{Na}^+$ ,  $\text{K}^+/\text{Cl}^-$  cotransporter, accumulating in the cell above its electrochemical equilibrium concentration. Secretion of  $\text{Cl}^-$  from the apical surface, in response to hormonal stimulation, leads to flow of  $\text{Na}^+$  and water into the secretory lumen. The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel encoded by the gene for cystic fibrosis, a common fatal genetic disorder in humans. Loss of CFTR function decreases transepithelial water secretion and, as a result, the layers of mucus that coat the respiratory tree, pancreatic ducts, and intestine are dehydrated and difficult to clear. The resulting blockage of these sites leads to pancreatic insufficiency, "meconium ileus," and devastating "chronic obstructive pulmonary disease" (Al-Awqati, Q. et al. (1992) J. Exp. Biol. 172:245-266).

Many intracellular organelles contain  $\text{H}^+$ -ATPase pumps that generate transmembrane pH and electrochemical differences by moving protons from the cytosol to the organelle lumen. If the membrane of the organelle is permeable to other ions, then the electrochemical gradient can be abrogated without affecting the pH differential. In fact, removal of the electrochemical barrier allows more  $\text{H}^+$  to be pumped across the membrane, increasing the pH differential.  $\text{Cl}^-$  is the sole counterion of  $\text{H}^+$  translocation in a number of organelles, including chromaffin granules, Golgi vesicles, lysosomes, and endosomes. Functions that require a low vacuolar pH include uptake of small molecules such as biogenic amines in chromaffin granules, processing of vacuolar constituents such as pro-hormones by proteolytic enzymes, and protein degradation in lysosomes (Al-Awqati, *supra*).

Ligand-gated channels open their pores when an extracellular or intracellular mediator binds to the channel. Neurotransmitter-gated channels are channels that open when a neurotransmitter binds to their extracellular domain. These channels exist in the postsynaptic

membrane of nerve or muscle cells. There are two types of neurotransmitter-gated channels. Sodium channels open in response to excitatory neurotransmitters, such as acetylcholine, glutamate, and serotonin. This opening causes an influx of  $\text{Na}^+$  and produces the initial localized depolarization that activates the voltage-gated channels and starts the action potential. Chloride channels open in response to inhibitory neurotransmitters, such as  $\gamma$ -aminobutyric acid (GABA) and glycine, leading to hyperpolarization of the membrane and the subsequent generation of an action potential.

Ligand-gated channels can be regulated by intracellular second messengers. Calcium-activated  $\text{K}^+$  channels are gated by internal calcium ions. In nerve cells, an influx of calcium during depolarization opens  $\text{K}^+$  channels to modulate the magnitude of the action potential (Ishi, T.M. et al. (1997) Proc. Natl. Acad. Sci. USA 94:11651-11656). Cyclic nucleotide-gated (CNG) channels are gated by cytosolic cyclic nucleotides. The best examples of these are the cAMP-gated  $\text{Na}^+$  channels involved in olfaction and the cGMP-gated cation channels involved in vision. Both systems involve ligand-activation of a G-protein coupled receptor which then alters the level of cyclic nucleotide within the cell. In olfaction, binding of an odorant to the receptor activates adenylate cyclase, leading to a rise in cytosolic cAMP. The cAMP binds to the cAMP-gated  $\text{Na}^+$  channel causing an influx of  $\text{Na}^+$ , depolarization of the membrane, and initiation of a nerve impulse that travels along the axon to the brain. In vision, light activation of rhodopsin leads to activation of cGMP phosphodiesterase, which hydrolyzes cGMP. As a result, cytosolic cGMP levels drop, cGMP dissociates from cGMP-gated cation channels, and the channels close, resulting in hyperpolarization of the membrane. (See Zagotta, W.M. and S.A. Siegelbaum (1996) Annu. Rev. Neurosci. 19:235-263; Molday, R.S. and L.L. Molday (1998) Vision Res. 38:1315-1323.)

The subunits or monomers of an ion channel may be identical or different. CNG channels, for example, consist of  $\alpha$  and  $\beta$  subunits that differ from each other at the N-terminal cytoplasmic tail. The central pore formed by the barrel arrangement is lined by an antiparallel  $\beta$ -sheet, the pore (P) region, contained within each subunit. This region also contains information specifying the ion selectivity for the channel. In the case of  $\text{K}^+$  channels, a GYG tripeptide is involved in this selectivity (Ishi et al., *supra*). In voltage-gated channels, one of the transmembrane domains contains regularly spaced, positively charged amino acids that act as a voltage-sensor. In CNG channels, a region in the C-terminal cytoplasmic domain acts as a cyclic nucleotide binding site (Zagotta and Siegelbaum, *supra*). Ion channels also have a domain that functions in inactivation of the channel. In CNG  $\text{K}^+$  channels, the inactivation domain is on the N-terminal cytoplasmic tail of the  $\beta$ -subunit. This domain acts as a tethered ball to block ion flow through the pore. This domain is also expressed as a separate protein, a glutamic acid-rich protein



identity to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-18 and fragments thereof.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid  
5 sequence selected from the group consisting of SEQ ID NO:1-18 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-18 and fragments thereof.

The invention also provides a method for detecting a polynucleotide in a sample  
10 containing nucleic acids, the method comprising the steps of (a) hybridizing the complement of the polynucleotide sequence to at least one of the polynucleotides of the sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide in the sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

15 The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:19-36, and fragments thereof. The invention further provides an isolated and purified polynucleotide variant having at least 95% polynucleotide sequence identity to the polynucleotide sequence selected from the group consisting of SEQ ID NO:19-36 and fragments thereof. The invention also provides an  
20 isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:19-36 and fragments thereof.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the  
25 group consisting of SEQ ID NO:1-18 and fragments thereof. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide under conditions suitable for the expression of the polypeptide; and  
30 (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-18 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide selected

from the group consisting of SEQ ID NO:1-18 and fragments thereof. The invention also provides a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing a disorder associated with decreased expression or activity of MECHP, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-18 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention also provides a method for treating or preventing a disorder associated with increased expression or activity of MECHP, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-18 and fragments thereof.

#### BRIEF DESCRIPTION OF THE FIGURES AND TABLES

Figure 1 shows the amino acid sequence alignment between MECHP-1 (1568324; SEQ ID NO:1) and rat glutamic acid-rich protein (GI 2924369; SEQ ID NO:37), produced using the BLAST search tool.

Figure 2 shows the amino acid sequence alignment among MECHP-2 (4094907; SEQ ID NO:2), *Drosophila* voltage-gated potassium channel (GI 116443; SEQ ID NO:38), and *P. penicillatus* potassium channel  $\alpha$ -subunit (GI 1763619; SEQ ID NO:39), produced using the multisequence alignment program of LASERGENE software (DNASTAR, Madison WI).

Figures 3A and 3B show the amino acid sequence alignment between MECHP-3 (518158; SEQ ID NO:3) and rat calcium-activated potassium channel rSK3 (GI 2564072; SEQ ID NO:40), produced using the multisequence alignment program of LASERGENE software.

Figures 4A, 4B, and 4C show the amino acid sequence alignment among MECHP-4 (602926; SEQ ID NO:4), *Drosophila* voltage-gated potassium channel (GI 116443; SEQ ID NO:38) and *P. penicillatus* potassium channel  $\alpha$ -subunit (GI 1763619; SEQ ID NO:39), produced using the multisequence alignment program of LASERGENE software.

Figures 5A and 5B show the amino acid sequence alignment between MECHP-5 (922119; SEQ ID NO:5) and rat aquaporin 7 (GI 2350843; SEQ ID NO:41), produced using the multisequence alignment program of LASERGENE software.

Figures 6A and 6B show the amino acid sequence alignment between MECHP-7 (2731369; SEQ ID NO:7) and mouse connexin 30.3 (GI 192647; SEQ ID NO:42), produced using the multisequence alignment program of LASERGENE software.

Figure 7 shows the amino acid sequence alignment between MECHP-16 (2069907; SEQ ID NO:16) and human beta subunit of Ca<sup>2+</sup> activated K<sup>+</sup> channel (GI 1055345; SEQ ID NO:43), produced using the multisequence alignment program of LASERGENE software.

Figures 8A and 8B show the amino acid sequence alignment between MECHP-17 (2243917; SEQ ID NO:17) and a homolog of *Caenorhabditis elegans* K<sup>+</sup> channel protein (GI 3292929; SEQ ID NO:44), produced using the multisequence alignment program of LASERGENE software.

Figures 9A and 9B show the amino acid sequence alignment between MECHP-18 (2597476; SEQ ID NO:18) and human aquaporin 9 (GI 2887407; SEQ ID NO:45), produced using the multisequence alignment program of LASERGENE software.

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding MECHP.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods and algorithms used for identification of MECHP.

Table 3 shows the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis, diseases, disorders, or conditions associated with these tissues, and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding MECHP were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze MECHP, along with applicable descriptions, references, and threshold parameters.

## DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

## 10 DEFINITIONS

"MECHP" refers to the amino acid sequences of substantially purified MECHP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

15 The term "agonist" refers to a molecule which, when bound to MECHP, increases or prolongs the duration of the effect of MECHP. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of MECHP.

An "allelic variant" is an alternative form of the gene encoding MECHP. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

25 "Altered" nucleic acid sequences encoding MECHP include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as MECHP or a polypeptide with at least one functional characteristic of MECHP. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding MECHP, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding MECHP. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent MECHP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity,

hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of MECHP is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of MECHP which are preferably at least 5 to about 15 amino acids in length, most preferably at least 14 amino acids, and which retain some biological activity or immunological activity of MECHP. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which, when bound to MECHP, decreases the amount or the duration of the effect of the biological or immunological activity of MECHP. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of MECHP.

The term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind MECHP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on



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the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules  
5 may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

The term "biologically active" refers to a protein having structural, regulatory, or  
10 biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic MECHP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" and "complementarity" refer to the natural binding of  
15 polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of  
complementarity between nucleic acid strands has significant effects on the efficiency and strength  
20 of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" and a "composition  
comprising a given amino acid sequence" refer broadly to any composition containing the given  
25 polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding MECHP or fragments of MECHP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl),  
detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution,  
30 dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping

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sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

5 The term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding MECHP, by northern analysis is indicative of the presence of nucleic acids encoding MECHP in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding MECHP.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

10 The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any  
15 similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from  
20 hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target  
25 sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity).  
30 In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" and "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MEGALIGN program (DNASTAR)

which creates alignments between two or more sequences according to methods selected by the user, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) *Gene* 73:237-244.) Parameters for each method may be the default parameters provided by MEGALIGN or may be specified by the user. The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) *Methods Enzymol.* 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by varying hybridization conditions.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g.,  $C_0t$  or  $R_0t$  analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which

may affect cellular and systemic defense systems.

The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate.

The terms "element" and "array element" in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

5 The term "modulate" refers to a change in the activity of MECHP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of MECHP.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to  
10 DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which comprise a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:19-36, for example, as distinct from any other sequence in the same genome. For  
15 example, a fragment of SEQ ID NO:19-36 is useful in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:19-36 from related polynucleotide sequences. A fragment of SEQ ID NO:19-36 is at least about 15-20 nucleotides in length. The precise length of the fragment of SEQ ID NO:19-36 and the region of SEQ ID NO:19-36 to which the fragment corresponds are routinely determinable by one of ordinary skill  
20 in the art based on the intended purpose for the fragment. In some cases, a fragment, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

The terms "operably associated" and "operably linked" refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if  
25 the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

The term "oligonucleotide" refers to a nucleic acid sequence of at least about 6 nucleotides  
30 to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. "Oligonucleotide" is substantially equivalent to the terms "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which

comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

5       The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding MECHP, or fragments thereof, or MECHP itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

10       The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds  
15       to the antibody.

20       The term "stringent conditions" refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, e.g., formamide, temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

25       The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

      A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

30       "Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

      "Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of

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foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is

5 capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of MECHP polypeptides refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes, wherein a

10 substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using

15 computer programs well known in the art, for example, LASERGENE software (DNASTAR).

The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to MECHP. This definition may also include, for example, "allelic" (as defined above), "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or

20 lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between

25 individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

## THE INVENTION

30 The invention is based on the discovery of new human membrane channel proteins (MECHP), the polynucleotides encoding MECHP, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative, immune/inflammatory, transport/secretory, osmoregulatory, muscular, cardiovascular, and neurological disorders.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding

MECHP. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each MECHP were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. The clones in column 5 were used to assemble the consensus nucleotide sequence of each MECHP and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows the identity of each polypeptide; and column 7 shows analytical methods used to identify each polypeptide through sequence homology and protein motifs.

MECHP-1 has chemical and structural similarity with rat glutamic acid-rich protein (GI 2924369; SEQ ID NO:37). In particular, MECHP-1 and rat glutamic acid-rich protein share 15% overall identity. As shown in Figure 1, BLAST analysis identifies regions of MECHP-1 and rat glutamic acid-rich protein which share 27-30% identity. These regions extend from residue V12 through T163, P266 through G344, P461 through E548, and E653 through G709 in MECHP-1.

As shown in Figure 2, MECHP-2 has chemical and structural similarity with Drosophila voltage-gated potassium channel (GI 116443; SEQ ID NO:38) and P. penicillatus potassium channel  $\alpha$ -subunit (GI 1763619; SEQ ID NO:39). In particular, MECHP-2 shares 18% identity with Drosophila voltage-gated K<sup>+</sup> channel, and 17% identity with P. penicillatus K<sup>+</sup> channel  $\alpha$ -subunit. In particular, MECHP-2 shares 27% identity with Drosophila voltage-gated potassium channel and P. penicillatus potassium channel  $\alpha$ -subunit over the first 133 residues, from M1 through T133 in MECHP-2.

As shown in Figures 3A and 3B, MECHP-3 has chemical and structural similarity with rat calcium-activated potassium channel rSK3 (GI 2564072; SEQ ID NO:40). In particular, MECHP-3 and rat rSK3 share 40% identity. MECHP-3 and rat rSK3 also share a canonical ion pore (P) region, including a GYG potassium ion selectivity sequence, from residue W192 through G213 in MECHP-3.

As shown in Figures 4A, 4B, and 4C, MECHP-4 has chemical and structural similarity with Drosophila voltage-gated potassium channel (GI 116443; SEQ ID NO:38) and P. penicillatus potassium channel  $\alpha$ -subunit (GI 1763619; SEQ ID NO:39). In particular, MECHP-4 shares 28%

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identity with Drosophila voltage-gated K<sup>+</sup> channel, and 26% identity with P. penicillatus K<sup>+</sup> channel  $\alpha$ -subunit, respectively. MECHP-4, Drosophila voltage-gated K<sup>+</sup> channel, and P. penicillatus K<sup>+</sup> channel  $\alpha$ -subunit also share a GYG potassium ion selectivity sequence from residue G372 through G374 in MECHP-4.

5 As shown in Figures 5A and 5B, MECHP-5 has chemical and structural similarity with rat aquaporin 7 (GI 2350843; SEQ ID NO:41). In particular, MECHP-5 and rat aquaporin 7 share 74% identity.

As shown in Figures 6A and 6B, MECHP-7 has chemical and structural similarity with mouse connexin 30.3 (GI 192647; SEQ ID NO:42). In particular, MECHP-7 and mouse connexin  
10 30.3 (GI 192647) share 84% identity.

As shown in Figure 7, MECHP-16 has chemical and structural similarity with human beta subunit of Ca<sup>+</sup> activated K<sup>+</sup> channel (GI 1055345; SEQ ID NO:43). In particular, MECHP-16 and human beta subunit of Ca<sup>+</sup> activated K<sup>+</sup> channel share 40% identity.

As shown in Figures 8A and 8B, MECHP-17 has chemical and structural similarity with a  
15 homolog of C. elegans K<sup>+</sup> channel protein (GI 3292929; SEQ ID NO:44). In particular, MECHP-17 and the specified homolog of C. elegans K<sup>+</sup> channel protein share 47% identity.

As shown in Figures 9A and 9B, MECHP-18 has chemical and structural similarity with human aquaporin 9 (GI 2887407; SEQ ID NO:45). In particular, MECHP-18 and human aquaporin 9 share 46% identity.

20 The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding MECHP. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists tissue categories which express MECHP as a fraction of total tissue categories expressing MECHP. Column 3 lists diseases, disorders, or conditions associated with those tissues expressing MECHP. Column 4 lists the vectors used to subclone the  
25 cDNA library. Northern analysis shows the expression of SEQ ID NO:34 in only 7 libraries, of which 6 (86%) are associated with cell proliferation. Two of these libraries are associated with brain tissue, one with pancreatic islet cells, one with kidney tissue, one with fetal lung tissue, one with ovarian tissue, and one with adrenal tissue. Northern analysis shows the expression of SEQ ID NO:36 in only 3 libraries, one of which is associated with ovarian tumor tissue, one with  
30 developing lung tissue, and one with gastrointestinal tissue associated with inflammation. Of particular note is the enriched expression of MECHP in neural and neuroendocrine tissue, most prominently the neural tissue-specific expression of SEQ ID NO:30.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding MECHP were isolated. Column 1 references the



nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

The following fragments of the nucleotide sequences encoding MECHP are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:19-36, and to distinguish between SEQ ID NO:19-36 and related polynucleotide sequences. The useful fragments include the fragment of SEQ ID NO:19 from about nucleotide 764 to about nucleotide 808; the fragment of SEQ ID NO:20 from about nucleotide 523 to about nucleotide 582; the fragment of SEQ ID NO:21 from about nucleotide 628 to about nucleotide 669; the fragment of SEQ ID NO:22 from about nucleotide 779 to about nucleotide 826; the fragment of SEQ ID NO:23 from about nucleotide 64 to about nucleotide 108; the fragment of SEQ ID NO:24 from about nucleotide 1133 to about nucleotide 1180; the fragment of SEQ ID NO:25 from about nucleotide 656 to about nucleotide 700; the fragment of SEQ ID NO:26 from about nucleotide 153 to about nucleotide 197; the fragment of SEQ ID NO:27 from about nucleotide 2160 to about nucleotide 2219; the fragment of SEQ ID NO:28 from about nucleotide 1275 to about nucleotide 1322; the fragment of SEQ ID NO:29 from about nucleotide 313 to about nucleotide 348; the fragment of SEQ ID NO:30 from about nucleotide 994 to about nucleotide 1041; the fragment of SEQ ID NO:31 from about nucleotide 443 to about nucleotide 478; the fragment of SEQ ID NO:32 from about nucleotide 1175 to about nucleotide 1207; the fragment of SEQ ID NO:34 from about nucleotide 381 to about nucleotide 425; the fragment of SEQ ID NO:35 from about nucleotide 17 to about nucleotide 61; and the fragment of SEQ ID NO:36 from about nucleotide 54 to about nucleotide 98. The polypeptides encoded by the fragments of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:34, SEQ ID NO:35, AND SEQ ID NO:36 are useful, for example, as immunogenic peptides.

The invention also encompasses MECHP variants. A preferred MECHP variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the MECHP amino acid sequence, and which contains at least one functional or structural characteristic of MECHP.

The invention also encompasses polynucleotides which encode MECHP. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:19-36, which encodes MECHP.

The invention also encompasses a variant of a polynucleotide sequence encoding MECHP. In particular, such a variant polynucleotide sequence will have at least about 70%, more

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preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding MECHP. A particular aspect of the invention encompasses a variant of a sequence selected from the group consisting of SEQ ID NO:19-36 which has at least about 70%, more preferably at least about 85%, and most preferably at least  
5 about 95% polynucleotide sequence identity to a sequence selected from the group consisting of SEQ ID NO:19-36. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of MECHP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding MECHP, some bearing minimal  
10 similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring MECHP, and all such variations are to be  
15 considered as being specifically disclosed.

Although nucleotide sequences which encode MECHP and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring MECHP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding MECHP or its derivatives possessing a substantially different codon usage,  
20 e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding MECHP and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more  
25 desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode MECHP and MECHP derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell  
30 systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding MECHP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:19-36, or to a fragment of SEQ ID NO:19-36, under various conditions of stringency. (See,

e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or

combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the Robbins Hydra microdispenser (Robbins Scientific, Sunnyvale CA), Hamilton MICROLAB 2200 (Hamilton, Reno NV), Peltier Thermal  
5 Cycler 200 (PTC200; MJ Research, Watertown MA) and the ABI CATALYST 800 (Perkin-Elmer). Sequencing is then carried out using either ABI 373 or 377 DNA sequencing systems (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short  
10 Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding MECHP may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which  
15 may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See,  
20 e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR.  
25 Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available  
30 software, such as OLIGO 4.06 primer analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include

sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to  
 5 analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer),  
 10 and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode MECHP may be cloned in recombinant DNA molecules that direct expression of  
 15 MECHP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express MECHP.

The nucleotide sequences of the present invention can be engineered using methods  
 20 generally known in the art in order to alter MECHP-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create  
 25 new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding MECHP may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223, and Horn, T. et al. (1980) Nucleic Acids Symp. Ser.  
 30 7:225-232.) Alternatively, MECHP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of MECHP, or any part thereof, may be altered during direct synthesis and/or

combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

In order to express a biologically active MECHP, the nucleotide sequences encoding MECHP or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding MECHP. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding MECHP. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding MECHP and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding MECHP and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding MECHP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression

vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not  
5 limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding MECHP. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding MECHP can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla  
10 CA) or pSPORT1 plasmid (Life Technologies). Ligation of sequences encoding MECHP into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G.  
15 and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of MECHP are needed, e.g. for the production of antibodies, vectors which direct high level expression of MECHP may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of MECHP. A number of vectors  
20 containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al.  
25 (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of MECHP. Transcription of sequences encoding MECHP may be driven by viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or  
30 heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding MECHP may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses MECHP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of MECHP in cell lines is preferred. For example, sequences encoding MECHP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* or *apr* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech),  $\beta$  glucuronidase and its



substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) *Methods Mol. Biol.* 55:121-131.)

5 Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding MECHP is inserted within a marker gene sequence, transformed cells containing sequences encoding MECHP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence  
10 encoding MECHP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding MECHP and that express MECHP may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR  
15 amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of MECHP using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such  
20 techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on MECHP is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical  
25 Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled  
30 hybridization or PCR probes for detecting sequences related to polynucleotides encoding MECHP include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding MECHP, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an

appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, 5 fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding MECHP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on 10 the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode MECHP may be designed to contain signal sequences which direct secretion of MECHP through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications 15 of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from 20 the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding MECHP may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric MECHP 25 protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of MECHP activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding 30 peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A

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fusion protein may also be engineered to contain a proteolytic cleavage site located between the MECHP encoding sequence and the heterologous protein sequence, so that MECHP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch 10). A variety of  
5 commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled MECHP may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract systems (Promega). These systems couple transcription and translation of protein-coding sequences  
10 operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, preferably <sup>35</sup>S-methionine.

Fragments of MECHP may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis  
15 may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Various fragments of MECHP may be synthesized separately and then combined to produce the full length molecule.

## THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists  
20 between regions of MECHP and human membrane channel proteins. In addition, the expression of MECHP is closely associated with nervous, reproductive, and gastrointestinal tissues; fetal development; and neurological, immune/inflammatory, and cell proliferative disorders, including cancer. Therefore, MECHP appears to play a role in cell proliferative, immune/inflammatory, transport/secretory, osmoregulatory, muscular, cardiovascular, and neurological disorders. In the  
25 treatment of disorders associated with increased MECHP expression or activity, it is desirable to decrease the expression or activity of MECHP. In the treatment of disorders associated with decreased MECHP expression or activity, it is desirable to increase the expression or activity of MECHP.

Therefore, in one embodiment, MECHP or a fragment or derivative thereof may be  
30 administered to a subject to treat or prevent a disorder associated with decreased expression or activity of MECHP. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma,

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leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an

5 immune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus,

10 emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma,

15 Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a transport/secretory disorder such as akinesia, amyotrophic lateral sclerosis, ataxia telangiectasia, cystic fibrosis, Becker's muscular dystrophy, Bell's palsy,

20 Charcot-Marie Tooth disease, Chediak-Higashi syndrome, diabetes mellitus, diabetes insipidus, diabetic neuropathy, hyperkalemic periodic paralysis, normokalemic periodic paralysis, malignant hyperthermia, multidrug resistance, myotonic dystrophy, catatonia, dystonias, peripheral neuropathy, neurofibromatosis, postherpetic neuralgia, trigeminal neuropathy, sarcoidosis, sickle cell anemia, toxic shock syndrome, Wilson's disease, cataracts, infertility, pulmonary artery

25 stenosis, sensorineural autosomal deafness, hyperglycemia, hypoglycemia, goiter, Cushing's disease, glucose-galactose malabsorption syndrome, hypercholesterolemia, and allergies, including hay fever, asthma, and urticaria (hives); an osmoregulatory disorder such as diabetes insipidus, diarrhea, peritonitis, chronic renal failure, Addison's disease, SIADH, hypoaldosteronism, hyponatremia, adrenal insufficiency, hypothyroidism, hypernatremia,

30 hypokalemia, Barter's syndrome, metabolic acidosis, metabolic alkalosis, encephalopathy, edema, hypotension, and hypertension; a muscular disorder such as cardiomyopathy, myocarditis, Duchenne's muscular dystrophy, Becker's muscular dystrophy, myotonic dystrophy, central core disease, nemaline myopathy, centronuclear myopathy, lipid myopathy, mitochondrial myopathy, infectious myositis, polymyositis, dermatomyositis, inclusion body myositis, thyrotoxic myopathy,

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and ethanol myopathy; a cardiovascular disorder such as arteriovenous fistula, atherosclerosis, hypertension, vasculitis, Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, vascular tumors, and complications of thrombolysis, balloon angioplasty, vascular replacement, and coronary artery bypass graft surgery; congestive heart failure, ischemic heart disease, angina pectoris, myocardial infarction, hypertensive heart disease, degenerative valvular heart disease, calcific aortic valve stenosis, congenitally bicuspid aortic valve, mitral annular calcification, mitral valve prolapse, rheumatic fever and rheumatic heart disease, infective endocarditis, nonbacterial thrombotic endocarditis, endocarditis of systemic lupus erythematosus, carcinoid heart disease, cardiomyopathy, myocarditis, pericarditis, neoplastic heart disease, congenital heart disease, complications of cardiac transplantation; congenital lung anomalies, atelectasis, pulmonary congestion and edema, pulmonary embolism, pulmonary hemorrhage, pulmonary infarction, pulmonary hypertension, vascular sclerosis, obstructive pulmonary disease, restrictive pulmonary disease, chronic obstructive pulmonary disease, emphysema, chronic bronchitis, bronchial asthma, bronchiectasis, bacterial pneumonia, viral and mycoplasmal pneumonia, lung abscess, pulmonary tuberculosis, diffuse interstitial diseases, pneumoconioses, sarcoidosis, idiopathic pulmonary fibrosis, desquamative interstitial pneumonitis, hypersensitivity pneumonitis, pulmonary eosinophilia bronchiolitis obliterans-organizing pneumonia, diffuse pulmonary hemorrhage syndromes, Goodpasture's syndromes, idiopathic pulmonary hemosiderosis, pulmonary involvement in collagen-vascular disorders, pulmonary alveolar proteinosis, lung tumors, inflammatory and noninflammatory pleural effusions, pneumothorax, pleural tumors, drug-induced lung disease, radiation-induced lung disease, and complications of lung transplantation; and a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Down syndrome, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases; neuromuscular disorders including spinal

muscular atrophy, carpal tunnel syndrome, mononeuritis multiplex; muscular dystrophies such as Duchenne's, myotonic facioscapulohumeral, oculopharyngeal, scapuloperoneal, congenital, distal, and ocular; congenital and metabolic myopathies, myotonia, peripheral nervous system disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies;  
 5 myasthenia gravis, periodic paralysis; mental disorders including depression and bipolar disorder, and mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; abnormalities in electrolytes such as calcium, phosphate, magnesium, and potassium; hypo- and hyperfunction of the thyroid, adrenal,  
 10 parathyroid, and pituitary; and primary and metastatic neoplasms.

In another embodiment, a vector capable of expressing MECHP or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of MECHP including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially  
 15 purified MECHP in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of MECHP including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of MECHP may be administered to a subject to treat or prevent a disorder associated with decreased expression or  
 20 activity of MECHP including, but not limited to, those listed above.

In a further embodiment, an antagonist of MECHP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of MECHP. Such disorders may include, but are not limited to, those discussed above. In one aspect, an antibody which specifically binds MECHP may be used directly as an antagonist or indirectly as a targeting  
 25 or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express MECHP.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding MECHP may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of MECHP including, but not limited to, those described above.

30 In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment

or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

5 An antagonist of MECHP may be produced using methods which are generally known in the art. In particular, purified MECHP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind MECHP. Antibodies to MECHP may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e.,  
10 those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with MECHP or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not  
15 limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to  
20 MECHP have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of MECHP amino acids may be fused with those of another protein, such as KLH, and antibodies to  
25 the chimeric molecule may be produced.

Monoclonal antibodies to MECHP may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al.  
30 (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984)

Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce MECHP-specific single chain antibodies. Antibodies with related specificity, but of distinct  
5 idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents  
10 as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for MECHP may also be generated. For example, such fragments include, but are not limited to, F(ab')<sub>2</sub> fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing  
15 the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays  
20 using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between MECHP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering MECHP epitopes is preferred, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for MECHP. Affinity is expressed as an association constant,  $K_a$ , which is defined as the molar concentration of MECHP-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The  $K_a$  determined for a preparation of polyclonal antibodies, which are  
25 heterogeneous in their affinities for multiple MECHP epitopes, represents the average affinity, or avidity, of the antibodies for MECHP. The  $K_a$  determined for a preparation of monoclonal antibodies, which are monospecific for a particular MECHP epitope, represents a true measure of affinity. High-affinity antibody preparations with  $K_a$  ranging from about  $10^9$  to  $10^{12}$  l/mole are  
30 preferred for use in immunoassays in which the MECHP-antibody complex must withstand



rigorous manipulations. Low-affinity antibody preparations with  $K_a$  ranging from about  $10^6$  to  $10^7$  l/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of MECHP, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is preferred for use in procedures requiring precipitation of MECHP-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding MECHP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding MECHP may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding MECHP. Thus, complementary molecules or fragments may be used to modulate MECHP activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding MECHP.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding MECHP. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

Genes encoding MECHP can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding MECHP. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing



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and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

5 Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al.  
10 (1997) Nat. Biotech. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a  
15 pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of MECHP, antibodies to MECHP, and mimetics, agonists, antagonists, or inhibitors of MECHP. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible  
20 pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial,  
25 intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used  
30 pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets,

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pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after  
5 grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired,  
10 disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or  
15 solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or  
20 starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution,  
25 Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or  
30 liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acids. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of MECHP, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example MECHP or fragments thereof, antibodies of MECHP, and agonists, antagonists or inhibitors of MECHP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the  $ED_{50}$  (the dose therapeutically effective in 50% of the population) or  $LD_{50}$  (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the  $LD_{50}/ED_{50}$  ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the  $ED_{50}$  with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1  $\mu\text{g}$  to 100,000  $\mu\text{g}$ , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

## 15 **DIAGNOSTICS**

In another embodiment, antibodies which specifically bind MECHP may be used for the diagnosis of disorders characterized by expression of MECHP, or in assays to monitor patients being treated with MECHP or agonists, antagonists, or inhibitors of MECHP. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics.

20 Diagnostic assays for MECHP include methods which utilize the antibody and a label to detect MECHP in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

25 A variety of protocols for measuring MECHP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of MECHP expression. Normal or standard values for MECHP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to MECHP under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of MECHP expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding MECHP may be

used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of MECHP may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of MECHP, and to monitor regulation of MECHP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding MECHP or closely related molecules may be used to identify nucleic acid sequences which encode MECHP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding MECHP, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the MECHP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:19-36 or from genomic sequences including promoters, enhancers, and introns of the gene encoding MECHP.

Means for producing specific hybridization probes for DNAs encoding MECHP include the cloning of polynucleotide sequences encoding MECHP or MECHP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding MECHP may be used for the diagnosis of disorders associated with expression of MECHP. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas,

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- parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an immune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune
- 5 polynodocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis,
- 10 myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and
- 15 helminthic infections, and trauma; a transport/secretory disorder such as akinesia, amyotrophic lateral sclerosis, ataxia telangiectasia, cystic fibrosis, Becker's muscular dystrophy, Bell's palsy, Charcot-Marie Tooth disease, Chediak-Higashi syndrome, diabetes mellitus, diabetes insipidus, diabetic neuropathy, hyperkalemic periodic paralysis, normokalemic periodic paralysis, malignant hyperthermia, multidrug resistance, myotonic dystrophy, catatonia, dystonias, peripheral
- 20 neuropathy, neurofibromatosis, postherpetic neuralgia, trigeminal neuropathy, sarcoidosis, sickle cell anemia, toxic shock syndrome, Wilson's disease, cataracts, infertility, pulmonary artery stenosis, sensorineural autosomal deafness, hyperglycemia, hypoglycemia, goiter, Cushing's disease, glucose-galactose malabsorption syndrome, hypercholesterolemia, and allergies, including hay fever, asthma, and urticaria (hives); an osmoregulatory disorder such as diabetes
- 25 insipidus, diarrhea, peritonitis, chronic renal failure, Addison's disease, SIADH, hypoaldosteronism, hyponatremia, adrenal insufficiency, hypothyroidism, hypernatremia, hypokalemia, Barter's syndrome, metabolic acidosis, metabolic alkalosis, encephalopathy, edema, hypotension, and hypertension; a muscular disorder such as cardiomyopathy, myocarditis, Duchenne's muscular dystrophy, Becker's muscular dystrophy, myotonic dystrophy, central core
- 30 disease, nemaline myopathy, centronuclear myopathy, lipid myopathy, mitochondrial myopathy, infectious myositis, polymyositis, dermatomyositis, inclusion body myositis, thyrotoxic myopathy, and ethanol myopathy; a cardiovascular disorder such as arteriovenous fistula, atherosclerosis, hypertension, vasculitis, Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, vascular tumors, and complications of thrombolysis,





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dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including depression and bipolar disorder, and mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; abnormalities in electrolytes such as calcium, phosphate, magnesium, and potassium; hypo- and hyperfunction of the thyroid, adrenal, parathyroid, and pituitary; and primary and metastatic neoplasms. The polynucleotide sequences encoding MECHP may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered MECHP expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding MECHP may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding MECHP may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding MECHP in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of MECHP, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding MECHP, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period

ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or over-expressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding MECHP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding MECHP, or a fragment of a polynucleotide complementary to the polynucleotide encoding MECHP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of MECHP include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding MECHP may be used to generate hybridization probes useful in mapping the naturally occurring genomic

sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding MECHP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, MECHP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between MECHP and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test

compounds are synthesized on a solid substrate. The test compounds are reacted with MECHP, or fragments thereof, and washed. Bound MECHP is then detected by methods well known in the art. Purified MECHP can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding MECHP specifically compete with a test compound for binding MECHP. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with MECHP.

In additional embodiments, the nucleotide sequences which encode MECHP may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. [Attorney Docket No. PF-0589 P, filed September 2, 1998], U.S. Ser. No. [Attorney Docket No. PF-0632 P, filed November 12, 1998], U.S. Ser. No. [Attorney Docket No. PF-0648 P, filed December 9, 1998], U.S. Ser. No. [Attorney Docket No. PF-0664 P, filed January 26, 1999], and U.S. Ser. No. [Attorney Docket No. PF-0671 P, filed February 10, 1999], are hereby expressly incorporated by reference.

## EXAMPLES

### I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA

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purity. In some cases, RNA was treated with DNase. For most libraries, poly(A<sup>+</sup>) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the

5 POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel,

10 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative  
15 agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), pSPORT1 plasmid (Life Technologies), or pINCY (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 $\alpha$ , DH10B, or ElectroMAX DH10B from Life Technologies.

## 20 II. Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision, using the UNIZAP vector system (Stratagene) or cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8  
25 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and  
30 thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a Fluoroskan II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

## III. Sequencing and Analysis

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cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing systems (Perkin-Elmer) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, *supra*, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example V.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open

reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:19-36. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

#### IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel, 1995, *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals, Palo Alto CA). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding MECHP occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer,



inflammation/trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in the description of the invention.

5    **V.      Extension of MECHP Encoding Polynucleotides**

The full length nucleic acid sequences of SEQ ID NO:19-36 was produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed  
10    using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one  
15    extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing  $Mg^{2+}$ ,  $(NH_4)_2SO_4$ , and  $\beta$ -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech),  
20    ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2  
25    min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100  $\mu$ l PICO GREEN quantitation reagent (0.25% (v/v) PICO GREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5  $\mu$ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was  
30    scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates,

digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with *Pfu* DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulphoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequence of SEQ ID NO:19-36 is used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

#### VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:19-36 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 µCi of [<sup>32</sup>P]-adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10<sup>7</sup> counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon

membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography and compared.

5 **VII. Microarrays**

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by  
10 hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

15 Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are  
20 arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schemm, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

25 **VIII. Complementary Polynucleotides**

Sequences complementary to the MECHP-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring MECHP. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides  
30 are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of MECHP. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the MECHP-encoding transcript.

## IX. Expression of MECHP

Expression and purification of MECHP are achieved using bacterial or virus-based expression systems. For expression of MECHP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express MECHP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of MECHP in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding MECHP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, MECHP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from MECHP at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch 10 and 16). Purified MECHP obtained by these methods can be used directly in the following activity assay.

## X. Demonstration of MECHP Activity

### Aquaporin Activity of MECHP

Aquaporin activity of MECHP is demonstrated as the ability to induce osmotic water permeability in Xenopus laevis oocytes injected with MECHP cRNA (Ishibashi, K. et al. (1994)

Proc. Natl. Acad. Sci. USA 91:6269-6273). Oocytes injected with water are used as the control. Injected oocytes are given a hypotonic shock by being transferred from 200 mosM to 70 mosM modified Barth's buffer. The increase in osmotic volume of the oocytes, observed at 24°C by videomicroscopy, is proportional to the MECHP aquaporin activity in the injected oocytes.

5 Protein Transport Activity of MECHP

Protein transport activity of MECHP is demonstrated by its ability to catalyze the translocation of newly synthesized preprolactin into proteoliposomes in an in vitro system (Görllich, D. and T.A. Rapoport (1993) Cell 75:615-630). Proteoliposomes are prepared containing purified MECHP, purified dog Sec61p beta and gamma, purified dog SRP receptor,  
10 and a mixture of phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol) corresponding approximately to those found in native microsomes. The proteoliposomes are incubated in a wheat germ in vitro translation system in which a secretory protein (preprolactin) is synthesized in the presence of SRP and radioactive amino acids. After translation and synthesis of preprolactin, half of the sample is treated with 500  
15 µg/ml proteinase K while the other half remains untreated. Any translocated preprolactin will be inaccessible to proteinase K while any untranslocated preprolactin will be degraded. The amount of preprolactin in the samples with and without proteinase K treatment is determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by phosphor image analysis. The amount of preprolactin protected from proteinase K digestion in the proteinase K-treated sample is  
20 proportional to the protein transport activity of MECHP.

Gap Junction Activity of MECHP

Gap junction activity of MECHP is demonstrated as the ability to induce the formation of intercellular channels between paired Xenopus laevis oocytes injected with MECHP cRNA (Hennemann, supra). One week prior to the experimental injection with MECHP cRNA, oocytes  
25 are injected with antisense oligonucleotide to MECHP to reduce background. MECHP cRNA-injected oocytes are incubated overnight, stripped of vitelline membranes, and paired for recording of junctional currents by dual cell voltage clamp. The measured conductances are proportional to gap junction activity of MECHP.

Ion Channel Activity of MECHP

30 Ion channel activity of MECHP is demonstrated using an electrophysiological assay for ion conductance. MECHP can be expressed by transforming a mammalian cell line such as COS7, HeLa or CHO with a eukaryotic expression vector encoding MECHP. Eukaryotic expression vectors are commercially available, and the techniques to introduce them into cells are well known to those skilled in the art. A second plasmid which expresses any one of a number of

marker genes, such as  $\beta$ -galactosidase, is co-transformed into the cells to allow rapid identification of those cells which have taken up and expressed the foreign DNA. The cells are incubated for 48-72 hours after transformation under conditions appropriate for the cell line to allow expression and accumulation of MECHP and  $\beta$ -galactosidase.

5 Transformed cells expressing  $\beta$ -galactosidase are stained blue when a suitable colorimetric substrate is added to the culture media under conditions that are well known in the art. Stained cells are tested for differences in membrane conductance due to potassium ions by electrophysiological techniques that are well known in the art. Untransformed cells, and/or cells transformed with either vector sequences alone or  $\beta$ -galactosidase sequences alone, are used as  
10 controls and tested in parallel. Cells expressing MECHP will have higher cation conductance relative to control cells. The contribution of MECHP to conductance can be confirmed by incubating the cells using antibodies specific for MECHP. The antibodies will bind to the extracellular side of MECHP, thereby blocking the pore in the ion channel, and the associated conductance.

15 Ion channel activity of MECHP is also measured as current flow across a MECHP-containing *Xenopus* oocyte membrane using the two-electrode voltage-clamp technique (Ishi et al., *supra*; Jegla, T. and L. Salkoff (1997) J. Neurosci. 17:32-44). MECHP is subcloned into an appropriate *Xenopus* oocyte expression vector, such as pBF, and 0.5-5 ng of mRNA is injected into mature stage IV oocytes. Injected oocytes are incubated at 18°C for 1-5 days. Inside-out  
20 macropatches are excised into an intracellular solution containing 116 mM K-gluconate, 4 mM KCl, and 10 mM Hepes (pH 7.2). The intracellular solution is supplemented with varying concentrations of the MECHP mediator, such as cAMP, cGMP, or  $\text{Ca}^{+2}$  (in the form of  $\text{CaCl}_2$ ), where appropriate. Electrode resistance is set at 2-5 M $\Omega$  and electrodes are filled with the intracellular solution lacking mediator. Experiments are performed at room temperature from a  
25 holding potential of 0 mV. Voltage ramps (2.5 s) from -100 to 100 mV are acquired at a sampling frequency of 500 Hz. Current measured is proportional to the activity of MECHP in the assay.

#### **XI. Functional Assays**

MECHP function is assessed by expressing the sequences encoding MECHP at  
30 physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10  $\mu\text{g}$  of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or

hematopoietic origin, using either liposome formulations or electroporation. 1-2 µg of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP, and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of MECHP on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding MECHP and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding MECHP and other genes of interest can be analyzed by northern analysis or microarray techniques.

## **XII. Production of MECHP Specific Antibodies**

MECHP substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the MECHP amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides 15 residues in length are synthesized using an ABI 431A peptide

synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity by, for example, binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

### **XIII. Purification of Naturally Occurring MECHP Using Specific Antibodies**

Naturally occurring or recombinant MECHP is substantially purified by immunoaffinity chromatography using antibodies specific for MECHP. An immunoaffinity column is constructed by covalently coupling anti-MECHP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing MECHP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of MECHP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/MECHP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and MECHP is collected.

### **XIV. Identification of Molecules Which Interact with MECHP**

MECHP, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton, A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled MECHP, washed, and any wells with labeled MECHP complex are assayed. Data obtained using different concentrations of MECHP are used to calculate values for the number, affinity, and association of MECHP with the candidate molecules.

25

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

30



Table 1

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	19	1568324	UTRSNOT05	668081H1 (SCORNOT01), 777659H1 (COLNNOT05), 1419526F1 (KIDNNOT09), 1568324F6 (UTRSNOT05), 1568324H1 (UTRSNOT05), 1691082F6 (PROSTUT10), 1866748F6 (SKINBIT01), 2133242H1 (ENDCNOT01), 2740849H1 (BRSTTUT14), 3488431H1 (EPIGNOT01), 3534835H1 (KIDNNOT25), 3556331H1 (LUNGNOT31), 3747086H1 (THYMNOT08)
2	20	4094907	BSCNSZT01	1298228F6 (BRSTNOT07), 1298228T6 (BRSTNOT07), 3518650T6 (LUNGNON03), 1673339T6 (BLADNOT05), 4094907H1 (BSCNSZT01), 810976R1 (LUNGNOT04)
3	21	518158	MMLR1DT01	518158H1 (MMLR1DT01), 1322305X302F1 (BLADNOT04), 1339742F1 (COLNTUT03), 1662883F6 (BRSTNOT09), 1868856F6 (SKINBIT01), 3329796H1 (HEAONOT04), SAPA00287F1
4	22	602926	BRSTTUT01	602926H1 (BRSTTUT01), 602926R1 (BRSTTUT01), 602926X15 (BRSTTUT01), 602926X18 (BRSTTUT01), 1236735H1 (LUNGNET03), 1294713F6 (PGANNCT03), 1342719X29R1 (COLNTUT03), 1796484T6 (PROSTUT05)
5	23	922119	RATRNOT02	922119H1 (RATRNOT02), 2304391T6 (BRSTNOT05), 2925760H1 (SININOT04), 2925760T6 (SININOT04), 3283088H1 (HEAONOT05), 5330728H1 (DRGTNON04), 5343411H1 (CONFNOT05)
6	24	2666782	THYMFET03	2666782H1 (THYMFET03), 2666782X305D2 (THYMFET03), 2666782X310F2 (THYMFET03), 2998445H1 (OVRTUT07), 2999052F6 (OVRTUT07), 3244028F6 (BRAINOT19), 3244028X317B2 (BRAINOT19)
7	25	2731369	OVRTUT04	2631755F6 (COLNTUT15), 2631755X300D1 (COLNTUT15), 2631755X303B1 (COLNTUT15), 2631755X303D1 (COLNTUT15), 2731369H1 (OVRTUT04), 2798719F6 (NPOLNOT01), 4406377H1 (PROSDIT01)

Table 1 (cont.)

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
8	26	1375415	LUNGNOT10	664378R6 (SCORNOT01), 1375415H1 (LUNGNOT10), 1477459H1 (CORPNOT02), 3068750H1 (UTRSNOR01)
9	27	2733282	OVRTUT04	1821059F6 (GBLATUT01), 2620171R6 (KERANOT02), 2620171X306U2 (KERANOT02), 2733282F6 (OVRTUT04), 2733282H1 (OVRTUT04), 2733282X315F1 (OVRTUT04), 3618886F6 (EPIENOT01), SBLA01906F1, SBLA01292F1
10	28	3148427	ADRENON04	3148427H1 (ADRENON04), 3877333F6 (HEARNOT06), 3877333T6 (HEARNOT06), 3877333X331B1 (HEARNOT06), 3877333X331U1 (HEARNOT06)
11	29	3342358	SPLNNOT09	259592X14 (HNT2RAT01), 2481052H1 (SMCANOT01), 3342358H1 (SPLNNOT09)
12	30	1267774	BRAINOT09	1267774F6 (BRAINOT09), 1267774H1 (BRAINOT09), 1740673R6 (HIPONON01), 1740673T6 (HIPONON01), 3242923H1 (BRAINOT19), 4837609H1 (BRAINOT01)
13	31	1817329	PROSNOT20	1817329F6 (PROSNOT20), 1817329H1 (PROSNOT20), 2506976F6 (CONUTUT01), 4313771F6 (BRAFNOT01), SAEA10065P1, SAEA02844F1, SAEA02136F1
14	32	3273307	PROSBPT06	2658420F6 (LUNGNOT09), 2658420X316D1 (LUNGNOT09), 2658420X325D1 (LUNGNOT09), 3273307F6 (PROSBPT06), 3273307H1 (PROSBPT06)
15	33	3824833	BRAXNOT01	3824833H1 (BRAXNOT01), SAGA02981F1, SAGA00581R1, SAGA01037F1
16	34	2069907	ISLTNOT01	2069907H1 (ISLTNOT01), 2069907X304D1 (ISLTNOT01), 2069907X313V1 (ISLTNOT01), 2736831F6 (OVARNOT09), 2736831T6 (OVARNOT09)
17	35	2243917	PANCTUT02	2243917H1 (PANCTUT02), 2243917F6 (PANCTUT02), 2108673R6 (BRAITUT03), 1804567F6 (SINTNOT13), 980106H1 (TONGTUT01)
18	36	2597476	OVRTUT02	2597476H1 (OVRTUT02), 2597476F6 (OVRTUT02), 1633918F6 (COLNNOT19), SAEC11415F1, SAEC10014F1, SBKA03380F1, SAEC10514F1

Table 2

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification	Analytical Methods
1	724	S123 S212 S246 S8 S20 S26 S32 T36 S38 T48 S73 S81 S82 S102 S161 S215 S227 S309 S375 T400 S492 S511 S545 S551 S564 S586 S627 T642 S119 S136 S152 S206 S240 S278 S424 S439 S444 S528 S539 S597 S607 T608 Y249	N582	ATP/GTP-binding site motif A (P-loop): A460-S467	Glutamic acid-rich protein (cyclic nucleotide-gated cation channel subunit)	BLAST MOTIFS
2	257	S162 S70 T93 T133 T242		Potassium channel signature: H74-T93	Potassium channel	MOTIFS PRINTS
3	377	S284 S174 S317 T101 T279 S338	N182 N334	Canonical ion pore region: W192-G213 Potassium channel signatures: T184-V206, G213-L239 Signal peptide: M1-S68 Transmembrane domains: W28-M46, I65-A81, L154-L173	Calcium-activated potassium channel	BLAST HMM MOTIFS PRINTS SPScan

Table 2 (cont.)

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification	Analytical Methods
4	491	S61 S144 S145 S150 S206 T274 S320 S463 S471 S472 T483 S75 T85 S118 T161 S183 T316 S489 Y437	N481 N487	Ion transport protein domain: C181-I405 Potassium channel signatures: E66-T85, P178-S206, G224-Q247, F250-L270, L294-S320, E323-E346, L354-T376, G383-F409 Potassium ion selectivity sequence: G372-G374 Transmembrane domain: V324-Y343	Voltage-gated potassium channel	BLAST HMM MOTIFS PFAM PRINTS
5	341	T189 S247 S9 Y292	N322	Major Intrinsic Protein (MIP): E26-Y271 Aquaporin NPA boxes: N93-A95, N225-S227 Transmembrane domain: M41-L59	Aquaporin	BLAST BLOCKS HMM MOTIFS PFAM PRINTS
6	476	T75 T105 T207 T222 S346 T378 S386 S71 T203 T224 S269 S309 Y235		Eubacterial secY protein: T75-I460 Signal peptide: M1-C46 Transmembrane domains: L33-F51, I147-L165, L239-I256	Sec61p alpha subunit	BLAST BLOCKS HMM MOTIFS PFAM PRINTS SPScan

Table 2 (cont.)

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification	Analytical Methods
7	266	S115 S121 T181	N119 N201	Connexin: M1-L208 Signal peptide: M1-A39 Transmembrane domain: I23-A39	Connexin	BLAST BLOCKS HMM MOTIFS ProfileScan PFAM PRINTS SPScan
8	182	S9 S130	N108	Transmembrane domains: R36-I55, A65-F84, V103-S130	Voltage-gated K <sup>+</sup> -channel (Plasmolipin)	BLAST HMM
9	942	S273 S302 S355 S368 T418 S419 S474 S498 T797 T2 T76 T92 S270 S318 S384 T557 T568 T593 T652 S685 T822 S823 S858 S927	N74 N97 N150 N231 N235 N253 N291 N521 N555 N579 N636 N821 N937	Signal peptide: M1-A31 Transmembrane domain: L900-L926	Calcium-dependent chloride channel (Lu-ECAM-1)	BLAST HMM SPScan
10	519	S74 S159 S187 T191 T224 T329 T441 S461 S466 S122 S172 T486 T516	N72 N215 N259 N394 N459	von Willebrand factor type A domain: D162-V337	L-type calcium channel subunit	BLAST PFAM PRINTS
11	251	S249 T52 T164 T182 S235 S35 S171	N50		Chloride intracellular channel (CLIC2)	BLAST

Table 2 (cont.)

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification	Analytical Methods
12	323	S173 T321 S44 S46 S165 S290 T50 S51 S169 T211 S228 S240	N48	PMP-22/PN20/EMP family protein: V8-Q207, T59-N72, Y176-D203 Signal peptide: M1-G29 Transmembrane domains: M10-V28, I106-A123, I134-S158, F180-V198	Voltage-gated Ca <sup>2+</sup> channel, gamma subunit	BLAST BLOCKS HMM MOTIFS PFAM SPScan
13	51	S5 T31			Ca <sup>2+</sup> channel, beta subunit	BLAST MOTIFS
14	113	S101 S102 T12 T46 T108 T65 S95 S96			Ca <sup>2+</sup> -activated K <sup>+</sup> channel	BLAST MOTIFS
15	215	T68 S106 S192 T198 T204	N42 N66 N74	Immunoglobulin domain: G43-I129 Myelin PO protein signature: L92-P119, D121-E150, A159-V183 Signal peptide: M1-S29 Transmembrane domain: T157-L177	Na <sup>+</sup> channel, beta subunit	BLAST HMM MOTIFS PFAM PRINTS SPScan

Table 2 (cont.)

SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification	Analytical Methods
16	235	T36 S90 S122 S176 S6 S12 T135	N88 N96 N119	Transmembrane domain: I48-T68	Ca <sup>2+</sup> -activated K <sup>+</sup> channel, beta subunit	BLAST HMM MOTIFS
17	234	S29 S82 T174 T216 T57 S221	N130	Microbodies C-terminal targeting signal: S232-M234	K <sup>+</sup> channel protein	BLAST MOTIFS
18	301	T47 S286	N75 N128 N133	MIP family protein domain: R15-Y260 MIP family signature: H80-A88 Microbodies C-terminal targeting signal: C299-L301 Transmembrane domain: N54-Y71	Aquaporin 9	BLAST HMM MOTIFS PFAM

Table 3

Nucleotide SEQ ID NO:	Tissue Expression (Fraction of Total)	Disease, Disorder or Condition (Fraction of Total)	Vector
19	Reproductive Fetal	Cell Proliferative (0.570) Immune Response (0.210)	pINCY
20	Nervous Reproductive	Cell Proliferative (0.560) Immune Response (0.320)	pINCY
21	Reproductive Hematopoietic/Immune Gastrointestinal	Cell Proliferative (0.660) Immune Response (0.370)	PSPORT1
22	Reproductive Gastrointestinal Musculoskeletal	Cell Proliferative (0.790) Immune Response (0.210)	PSPORT1
23	Gastrointestinal (0.333) Reproductive (0.292) Cardiovascular (0.208)	Cell Proliferative and Cancer (0.625) Inflammation (0.500)	PSPORT1
24	Nervous (0.455) Reproductive (0.409)	Cell Proliferative and Cancer (0.591) Inflammation (0.273) Neurological (0.182)	pINCY
25	Nervous (0.400) Reproductive (0.400) Gastrointestinal (0.200)	Cell Proliferative and Cancer (0.800) Inflammation (0.200)	pINCY
26	Nervous (0.533) Gastrointestinal (0.133)	Cell Proliferative (0.533) Inflammation (0.267) Neurological (0.113)	pINCY
27	Reproductive (0.333) Dermatologic (0.167) Gastrointestinal (0.167)	Cell Proliferative (0.750)	pINCY



Table 3 (cont.)

Nucleotide SEQ ID NO:	Tissue Expression (Fraction of Total)	Disease, Disorder or Condition (Fraction of Total)	Vector
28	Cardiovascular (0.250) Endocrine (0.250) Nervous (0.250)	Cancer (0.429) Trauma (0.429)	PSPORT1
29	Cardiovascular (0.500) Gastrointestinal (0.250) Hematopoietic/Immune (0.250)	Cancer (0.500) Inflammation (0.250)	pINCY
30	Nervous (1.000)	Inflammation (0.334) Neurological (0.167)	pINCY
31	Reproductive (0.500) Nervous (0.300) Gastrointestinal (0.200)	Cancer (0.600) Neurological (0.100)	pINCY
32	Reproductive (0.286) Endocrine (0.286)	Cancer (0.500) Inflammation (0.500)	pINCY
33	Nervous (0.667) Reproductive (0.333)	Cancer (0.333) Inflammation (0.333)	pINCY
34	Nervous (0.286)	Cell Proliferative (0.857)	pINCY
35	Nervous (0.296) Gastrointestinal (0.259) Reproductive (0.185)	Cell Proliferative (0.593) Inflammation/Immune Response (0.222)	pINCY
36	Reproductive (0.333) Cardiovascular (0.333) Gastrointestinal (0.333)	Cell Proliferative and Cancer (0.677) Inflammation (0.333)	pINCY

Table 4

Nucleotide SEQ ID NO:	Library	Library Comment
19	UTRSNOT05	Library was constructed using RNA isolated from the uterine tissue of a 45-year-old Caucasian female during a total abdominal hysterectomy and total colectomy. Pathology for the associated tumor tissue indicated multiple leiomyomas of the myometrium and a grade 2 colonic adenocarcinoma of the cecum. Patient history included multiple sclerosis and mitral valve disorder. Family history included type I diabetes, cerebrovascular disease, atherosclerotic coronary artery disease, malignant skin neoplasm, hypertension, and malignant neoplasm of the colon.
20	BSCNSZT01	Library was constructed using RNA isolated from diseased caudate nucleus tissue removed from the brain of a 49-year-old male. Patient history included schizophrenia.
21	MMLR1DT01	Library was constructed using RNA isolated from adherent mononuclear cells, which came from a pool of male and female donors. The cells were cultured for 24 hours following Ficoll Hypaque centrifugation.
22	BRSTTUT01	Library was constructed using RNA isolated from breast tumor tissue removed from a 55-year-old Caucasian female during a unilateral extended simple mastectomy. Pathology indicated invasive grade 4 mammary adenocarcinoma of mixed lobular and ductal type, extensively involving the left breast. Patient history included atrial tachycardia, blood in the stool, and a benign breast neoplasm. Family history included benign hypertension, atherosclerotic coronary artery disease, cerebrovascular disease, and depressive disorder.
23	RATRNOT02	Library was constructed using RNA isolated from the right atrium tissue of a 39-year-old Caucasian male, who died from a gunshot wound.
24	THYMFET03	Library was constructed using RNA isolated from thymus tissue removed from a Caucasian male fetus.

Table 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Comment
25	OVRTUT04	Library was constructed using RNA isolated from ovarian tumor tissue removed from a 53-year-old Caucasian female during a total abdominal hysterectomy, removal of the fallopian tubes and ovaries, regional lymph node excision, peritoneal tissue destruction, and incidental appendectomy. Pathology indicated grade 1 transitional cell carcinoma of the right ovary. The left ovary had a hemorrhagic corpus luteum. The uterus had multiple leiomyomas (1 submucosal, 11 intramural), and the endometrium was inactive. The cul-de-sac contained abundant histiocytes and rare clusters of mesothelial cells. Patient history included breast fibrosclerosis and chronic stomach ulcer. Family history included acute stomach ulcer with perforation, breast cancer, bladder cancer, rectal/anal cancer, benign hypertension, coronary angioplasty, and hyperlipidemia.
26	LUNGNOT10	Library was constructed using RNA isolated from lung tissue of a Caucasian male fetus, who died at 23 weeks' gestation.
27	OVRTUT04	Library was constructed using RNA isolated from ovarian tumor tissue removed from a 53-year-old Caucasian female during a total abdominal hysterectomy, removal of the fallopian tubes and ovaries, regional lymph node excision, and peritoneal tissue destruction. Pathology indicated grade 1 transitional cell carcinoma of the right ovary. The left ovary had a hemorrhagic corpus luteum. The uterus had multiple leiomyomas (1 submucosal, 11 intramural), and the endometrium was inactive. The cul-de-sac contained abundant histiocytes and rare clusters of mesothelial cells. Patient history included abundant fibrosclerosis and chronic stomach ulcer. Family history included acute stomach ulcer with perforation, breast cancer, bladder cancer, rectal/anal cancer, benign hypertension, coronary angioplasty, and hyperlipidemia.
28	ADRENON04	Library was constructed from 1.36 million independent clones from an adrenal tissue library. Starting RNA was made from adrenal gland tissue removed from a 20-year-old Caucasian male. The library was normalized in two rounds using conditions adapted from Soares et al. (Proc. Natl. Acad. Sci. USA (1994) 91:9228-9232) and Bonaldo et al. (Genome Res. (1996) 6:791-806), using a significantly longer (48-hours/round) reannealing hybridization period.

Table 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Comment
29	SPLNNOT09	Library was constructed using RNA isolated from diseased spleen tissue removed from a 22-year-old Caucasian male (Ashkenazi Jewish descent) during a total splenectomy. Pathology indicated Gaucher's disease with marked splenomegaly. Patient history included thyroid disorders and type 1 Gaucher's disease. Family history included benign hypertension, thyroid disease, myocardial infarction, cerebrovascular disease, arteriosclerotic cardiovascular disease, and prostate cancer.
30	BRAINOT09	Library was constructed using RNA isolated from brain tissue removed from a Caucasian male fetus, who died at 23 weeks' gestation.
31	PROSNOT20	Library was constructed using RNA isolated from diseased prostate tissue removed from a 65-year-old Caucasian male during a radical prostatectomy. Pathology indicated adenofibromatous hyperplasia. Pathology for the associated tumor tissue indicated an adenocarcinoma.
32	PROSBPT06	Library was constructed using RNA isolated from diseased prostate tissue removed from a 66-year-old Caucasian male during a radical prostatectomy and lymph node excision. Pathology indicated adenofibromatous hyperplasia. Pathology for the associated tumor tissue indicated grade 2 (of 4) adenocarcinoma, Gleason grade 3+3. The patient presented with elevated prostate specific antigen (PSA), proteinuria, decreased renal function, and urinary frequency. Patient history included hemiparesis, depressive disorder, sleep apnea, psoriasis, mitral valve prolapse, cerebrovascular disease, benign hypertension, and impotence. Family history included benign hypertension, cerebrovascular disease, and colon cancer.
33	BRAXNOT01	Library was constructed using RNA isolated from cerebellar tissue removed from a 70-year-old male. Patient history included chronic obstructive airways disease and left ventricular failure.
34	ISLTNOT01	Library was constructed using RNA isolated from a pooled collection of pancreatic islet cells.

Table 4 (cont.)

Nucleotide SEQ ID NO:	Library	Library Comment
35	PANCTUT02	Library was constructed using RNA isolated from pancreatic tumor tissue removed from a 45-year-old Caucasian female during radical pancreatic duodenectomy. Pathology indicated a grade 4 anaplastic carcinoma. Family history included benign hypertension, hyperlipidemia and atherosclerotic coronary artery disease.
36	OVRTUT02	Library was constructed using RNA isolated from ovarian tumor tissue removed from a 51-year-old Caucasian female during an exploratory laparotomy, total abdominal hysterectomy, salpingo-oophorectomy, and an incidental appendectomy. Pathology indicated mucinous cystadenoma presenting as a multiloculated neoplasm involving the entire left ovary. The right ovary contained a follicular cyst and a hemorrhagic corpus luteum. The uterus showed proliferative endometrium and a single intramural leiomyoma. The peritoneal biopsy indicated benign glandular inclusions consistent with endosalpingiosis. Family history included atherosclerotic coronary artery disease, benign hypertension, breast cancer, and uterine cancer.

Table 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	ESTs Probability value= 1.0E-8 or less Full Length sequences Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises as least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs fasta E value= 1.0E-6 Assembled ESTs. fasta Identity= 95% or greater and Match length=200 bases or greater, fastx E value= 1.0E-8 or less Full Length sequences fastx score= 100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS and PRINTS databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S and J.G. Henikoff, Nucl. Acid Res. 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105, and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and Probability value= 1.0E-3 or less where applicable
PFAM	A Hidden Markov Models-based application useful for protein family search.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits, depending on individual protein families

Table 5 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribnikov, M. et al. (1988) CABIOS 4:61-66; Gribnikov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al (1997) Nucleic Acids Res 25: 217-221.	Score= 40 or greater
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439	Score=5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. <u>supra</u> ; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

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What is claimed is:

1. A substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18, and fragments thereof.
2. A substantially purified variant having at least 95% amino acid sequence identity to the amino acid sequence of claim 1.
3. An isolated and purified polynucleotide encoding the polypeptide of claim 1.
4. An isolated and purified polynucleotide variant having at least 95% polynucleotide sequence identity to the polynucleotide of claim 3.
5. An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of claim 3.
6. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide of claim 3.
7. A method for detecting a polynucleotide, the method comprising the steps of:
  - (a) hybridizing the polynucleotide of claim 6 to at least one nucleic acid in a sample, thereby forming a hybridization complex; and
  - (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of the polynucleotide in the sample.
8. The method of claim 7 further comprising amplifying the polynucleotide prior to hybridization.
9. An isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:19-36 and fragments thereof.



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10. An isolated and purified polynucleotide variant having at least 95% polynucleotide sequence identity to the polynucleotide of claim 9.

11. An isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide of claim 9.

12. An expression vector comprising at least a fragment of the polynucleotide of claim 3.

13. A host cell comprising the expression vector of claim 12.

10

14. A method for producing a polypeptide, the method comprising the steps of:

(a) culturing the host cell of claim 13 under conditions suitable for the expression of the polypeptide; and

(b) recovering the polypeptide from the host cell culture.

15

15. A pharmaceutical composition comprising the polypeptide of claim 1 in conjunction with a suitable pharmaceutical carrier.

16. A purified antibody which specifically binds to the polypeptide of claim 1.

20

17. A purified agonist of the polypeptide of claim 1.

18. A purified antagonist of the polypeptide of claim 1.

25

19. A method for treating or preventing a disorder associated with decreased expression or activity of MECHP, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 15.

30

20. A method for treating or preventing a disorder associated with increased expression or activity of MECHP, the method comprising administering to a subject in need of such treatment an effective amount of the antagonist of claim 18.

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- (71) Applicant (for all designated States except US): INCYTE PHARMACEUTICALS, INC. [US/US]; 3160 Porter Drive, Palo Alto, CA 94304 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): AU-YOUNG,

[Continued on next page]

(54) Title: HUMAN MEMBRANE CHANNEL PROTEINS

12 VEPDPEAGSEQEVFSAVEGSPSAEETPSDITESPEVLETQLDAHQGLGMDPFGDMVDFVAA 1568324  
V P P ++ A P E P PE E Q + +P + AA

8 VLPQPGTPTQKTEEGAGFQPEITESKPEANFQPEP-EVQPEPEPEP-EPEPEPEPAPEEAA GI 2924369

72 ESTEDL---KALSSEEEEMGGAQEPESLLPPSVLDQASVIAERFVSSFSRRSS-VAQE 1568324  
+ L + + E+ E G + QE + PP QA V V +R SS +

66 PEVQTLPEEPFVEGEDVAEAGPSLQETQFADPQPTSQQAQA---VVKVNRPSWMLSW GI 2924369

128 DSKSSGFGSPRLVSRSSSVLSLEGSEKGLARHGSAT 1568324  
K P+ V SS +L E G + G+ T

122 FWKGMKVVLPQFVYSSSGGQNLAGEGGPDQDGAQT GI 2924369

461 PERDCKSPTVFCLOEAGEPLGKGKGRK-----FVLSLFDYEQDMAQE--HSPPKPSAG 1568324  
P++DG PC + G G K P L L + +L ++ PP PS A

150 PDQDGAQTLEFGTGDFGSEDGSDKTSKTQDTEPSLWLLRWLEINLEKVLQPPITPSQAW GI 2924369

514 EMSQRFFFNP-PAVSQRTTSPGGRPSARSPLSPTE 1568324  
++ P+ P P + P PS +P PE

210 KVEPEGAVLEFPDPTGTFMEVEPTENPSQNP-GFVE GI 2924369

653 EKGPLPSPTAGLEESSGQGPSPVALLGQVQDFQOSABQPKKEGSRDPADPSQGG 1568324  
E+ P P G + SS P FV L+ + + A QP G +PS G

246 EEEPAEPPQGFQASSLPPFGDPVRLIEWLLHRLMALPQFVLHCKAAEQEPSCTG GI 2924369

(57) Abstract: The invention provides new human membrane channel proteins (MECHP) and polynucleotides which identify and encode MECHP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of MECHP.

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12 VEPDPEAGSEQEVFSAVEGPSAEETPSDIESPEVLEIQLDHQGLIGMDPPGDMVDFVAA 1568324  
 V P P ++ A P E P PE E Q + +P + AA  
 8 VLPQPPGTPQKTEEGAGPQPETESKPEANRQPEP-EVQPEPEPEP-EPEPEPEPAPEFAA GI 2924369  
 72 ESTEDL---KALSSEEEEEEMGAAQEPESLPPSVLDQASVIAERFVSSFRRSS-VAQE 1568324  
 + L + + E+ E G + QE + PP QA V V +R SS +  
 66 PEVQIILPPEEPVEGEDVAEAGPSLQETQFADPPQPTSQAQVA-----VKVNRPSSWMLSW GI 2924369  
 128 DSKSSGFGSPRLVSRSSSVLSLEGSEKGLARHGSAT 1568324  
 K P+ V SS +L E G + G+ T  
 122 FWKGMKAVWPQVYSSGGQNLAAEGGPDQDGAQT GI 2924369  
 461 PERDGKSPVPCLOEEAGETPLGKGKRK-----PVLSLFDVEQIMAQE--HSPPKPSSAG 1568324  
 P++DG PC + G G K P L L + +L ++ PP PS A  
 150 PDQDGAQITLFPQIGIGDPGSEDKTSKTQDTEPSLWLLRWLEINLEKVLFPQPTPSQAW GI 2924369  
 514 EMSPQRRFFNP-PAVSQRTTSPGGRPSARSPISPT 1568324  
 ++ P+ P P + P PS +P P E  
 210 KVEPEGAVLEFPDPFGTMEVEPTENPSQPNP-GPVE GI 2924369  
 653 EKGPLPSPTAGLEESSGQGPSSPVALLGQVQDFQQAECQPKKEGSRDPADPSQQG 1568324  
 E+ P P G + SS P PV L+ + A QP G +PS G  
 246 EEEPAAEFPQGFQASSLPFGDPVRLIEWLLHRLMALPQFVLHGKAAEQEPSCPG GI 2924369

FIGURE 1

1 MSRP L I T R S P A S - - - - - P L N N Q G I P T P 4094907  
 1 M A S - V A A W L P F A R A A A I G W V P I A T H P L P P P G I 116443  
 1 M N G D I G A W I S C A R T A G I G W V P I S S K E - P S A G I 1763619  
 23 - - - - - A Q L T K S N A P V H I D V G G H M Y T S S L 4094907  
 30 P M P K D - - - R R K T D D E K L L I N V S G R R F E T W R G I 116443  
 30 Y L N K Q V C N E N E K N N A K L T I N V S G R R Y Q T Y S G I 1763619  
 46 A T L T K Y P E S R I G R L F D G T E P I V L - D S L K Q H 4094907  
 57 N T L E K Y P D T - - - L L G S N E R E F F Y D E D C K E G I 116443  
 60 H T L R K F K E T - - - L L G S Q E R D Y F Y D E S L E E G I 1763619  
 75 Y F I D R D G Q M F R Y I L N F L R T S K L L I P D D F K D 4094907  
 83 Y F F D R D P D I F R H I L N Y Y R T G K L H Y P K H - - E G I 116443  
 86 Y Y F D R D P D L F R H I L N Y Y R T G K L H F P K N - - E G I 1763619  
 105 Y T L L Y E E A - K Y F Q L Q P M L L E M E R W K Q D R E T 4094907  
 111 C L T S Y D E E L A F F G I M P D V I G D C C Y E D Y R D R G I 116443  
 114 C V S S F E D E L T F F G I K G F N I N N C C W D D Y H D K G I 1763619

FIGURE 2

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15 -- -- R K R L L E Q E K S L A G W A L V L A G T G I G L M V 518158  
271 L G H R R A L F E K R K R L S D Y A L I F G M F G I V V M V GI 2564072

42 L H A E M L W F G G C S W A L Y L F L V K C T I S I S T F L 518158  
301 I E T E L S W G L Y S K D S M F S L A L K C L I S L S T I I GI 2564072

72 L L C L I V A F H A K E V Q L F M T D N G L R D W R V A L T 518158  
331 L L G L I I A Y H T R E V Q L F V I D N G A D D W R I A M T GI 2564072

102 G R Q A A Q I V L E L V V C G L H P A P V R -- -- -- -- 518158  
361 Y E R I L Y I S L E M L V C A I H P I P G E Y K F F W T A R GI 2564072

124 -- -- -- -- G P P C V Q D L G A P L T S P Q P W P G F L G Q 518158  
391 L A F S Y T P S R A E A D V D I I L S I P M F L R L Y L I A GI 2564072

148 G E A L L S L A M L -- -- -- -- -- -- -- -- 518158  
421 R V M L L H S K L F T D A S S R S I G A L N K I N F N T R F GI 2564072

158 -- -- -- -- -- -- -- -- L L G L T L G L W L T T A W V L S 518158  
451 V M K T L M T I C P G T V L L V F S I S L W I I A A W T V R GI 2564072

175 V A E R -- -- Q A V N A T G H L S D T L W L I P I T F L T I G 518158  
481 V C E R Y H D Q Q D V T S N F L G A M W L I S I T F L S I G GI 2564072

FIGURE 3A

203 Y G D V V P G T M W G K I V C L C T G V M G V C C T A L L V 518158  
 511 Y G D M V P H T Y C G K G V C L L T G I M G A G C T A L L V GI 2564072  
  
 233 A V V A R K L E F N K A E K H V H N F M M D I Q Y T K E M K 518158  
 541 A V V A R K L E L T K A E K H V H N F M M D T Q L T K R I K GI 2564072  
  
 263 E S A A R V L Q E A W M F Y K H T R - - R K E S H A - A R R 518158  
 571 N A A A N V L R E T W L I Y K H T K L L K K I D H A K V R K GI 2564072  
  
 290 H Q R K L L A A I N A F R Q V R L K H R K L R E Q V N S M V 518158  
 601 H Q R K F L Q A I H Q L R G V K M E Q R K L S D Q A N T L V GI 2564072  
  
 320 D I S K M H M I L Y D L Q Q N L S S S H R A L E K Q I D T L 518158  
 631 D L S K M Q N V M Y D L I T E L N D R S E D L E K Q I G S L GI 2564072  
  
 350 A G K L D A L T - - - - - - - - - - - - - - - E L L 518158  
 661 E S K L E H L T A S F N S L P L L I A D T L R Q Q Q Q L L GI 2564072  
  
 361 S T A L G P R Q L P - - - - - - - - - - - - - - - E P S Q Q S K 518158  
 691 T A F V E A R G I S V A V G T S H A P P S D S P I G I S S T GI 2564072  
  
 377 518158  
 721 S F P T P Y T S S S S C GI 2564072

FIGURE 3B

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1 MV - - - - - FGEEFF - - - - - 602926  
 1 MNGDI GAWI SCARTAGI GWVP I SSK E - P S A GI 1763619  
 1 MAS - VAAWLPFAA AIGWVP I A THPLPP P GI 116443  
 8 - - - - - HRPQQDEELVNLNVGGFKQSV DQ 602926  
 30 YLNKQVCNENKNNAKLTINVS GRRYQ TYS GI 1763619  
 30 PMPK - - - - - DR RKT D E K L L I N V S G R R F E T W R GI 116443  
 31 S T L L R F P H T R L G K L L T C H S E E A I L E L C D D Y 602926  
 60 H T L R K F K E T - - - - - L L G S Q E R D Y F - - - - - Y GI 1763619  
 57 N T L E K Y P D T - - - - - L L G S N E R E F F - - - - - Y GI 116443  
 61 S V A D K E Y Y F D R N P S S F R Y V L N F Y Y T G K L H V 602926  
 80 D E S L E Y Y F D R D P D L F R H I L N Y Y R T G K L H F GI 1763619  
 77 D E D C K E Y F F D R D P D I F R H I L N Y Y R T G K L H Y GI 116443  
 91 M E E L C V F S F C Q E I E Y W G I N E L F I D S C C S N R 602926  
 110 P K N E C V S S F E D E L T F F G I K G F N I N N C C W D D GI 1763619  
 107 P K H E C L T S Y D E E L A F F G I M P D V I G D C C Y E D GI 116443  
 121 Y Q E R K E E N H E K D W D Q K S H D V S T D S S F E E S S 602926  
 140 Y H D K K R E C T E R L N E S D V M L T S S E I N E K S D T GI 1763619  
 137 Y R D R K R E N A E R L M D - - - - - - - - - - - D K GI 116443

FIGURE 4A

151 L F E K E L E K F D T L R F G Q L R K K I W I R M E N P A - 602926  
 170 M G I D V Q M N N H Q A K - - N F R Q K V H G L F E N P Q S GI 1763619  
 153 L S E N G D Q N L Q Q L T - - N M R Q K M W R A F E N P H T GI 116443  
 180 - - - - - Y C L S A K L I A I S S L S V V L A S I V A M 602926  
 198 T F L A R I L Y I T G F F I A V S V G S T I I E T I D - - GI 1763619  
 181 S T S A L V F Y Y V T G F F I A V S V M A N V V E T V P - - GI 116443  
 203 C V H S M S E F Q N E D - G E V D D P V L E G V E I A C I A 602926  
 226 C S A N R - - - - - P C G E V Y N K I F F N I E A V C V V GI 1763619  
 209 C G H R P G R A G T L P C G E R Y K I V F F C L D T A C V M GI 116443  
 232 W F T G E L A V R L A A A P C Q K K F W K N P L N I I D F V 602926  
 250 V F T I E Y L A R L Y S A P C R F R H A R I S L S I I D V I GI 1763619  
 239 I F T A E Y L L R L F A A P D R C K F V R S V M S I I D V V GI 116443  
 262 S I I P F Y A T L A V D T K E E E S E D I E N M G K V V Q I 602926  
 280 A I L P F Y I G L A M T - K T S I S G A F V S - - - - - GI 1763619  
 269 A I M P Y Y I G L G I T D N D D V S G A F V T - - - - - GI 116443  
 292 L R L M R I F R I L K L A R H S V G L R S L G A T L R H S Y 602926  
 302 L R V F R V F R I F K F S R H S K G L R I L G S T L T S C A GI 1763619  
 292 L R V F R V F R I F K F S R H S Q G L R I L G Y T L K S C A GI 116443

FIGURE 4B



322	H	E	V	G	L	L	L	F	L	S	V	G	I	S	I	F	S	V	L	I	Y	S	V	E	K	D	D	H	T	602926		
332	S	E	L	G	F	L	L	F	S	L	S	M	A	I	I	I	F	A	T	V	V	F	Y	V	E	K	D	V	N	D	GI 1763619	
322	S	E	L	G	F	L	L	V	F	S	L	A	M	A	I	I	I	F	A	T	V	M	F	Y	A	E	K	N	V	N	G	GI 116443
352	S	S	L	T	S	I	P	I	C	W	W	A	T	I	S	M	T	T	V	G	Y	G	D	T	H	P	V	T	L	602926		
362	S	D	F	T	S	I	P	A	S	F	W	Y	T	I	V	T	M	T	T	L	G	Y	G	D	M	V	P	K	T	I	GI 1763619	
352	T	N	F	T	S	I	P	A	A	F	W	Y	T	I	V	T	M	T	T	L	G	Y	G	D	M	V	P	E	T	I	GI 116443	
382	A	G	K	L	I	A	S	T	C	I	I	C	G	I	L	V	V	A	L	P	I	T	I	I	F	N	K	F	S	K	602926	
392	P	G	K	L	V	G	S	I	C	S	L	S	G	V	L	V	I	A	L	P	V	P	V	I	V	S	N	F	S	R	GI 1763619	
382	A	G	K	I	V	G	G	V	C	S	L	S	G	V	L	V	I	A	L	P	V	P	V	I	V	S	N	F	S	R	GI 116443	
412	Y	Y	Q	K	Q	K	D	I	D	V	D	Q	C	S	E	D	A	P	E	K	C	H	E	L	P	Y	F	N	I	R	602926	
422	I	Y	L	Q	N	Q	R	A	D	K	R	R	A	N	Q	K	L	R	-	-	-	-	-	-	N	K	C	E	E	K	GI 1763619	
412	I	Y	H	Q	N	Q	R	A	D	K	R	K	A	Q	R	K	A	R	L	A	R	I	R	I	A	K	A	S	S	G	GI 116443	
442	D	I	Y	A	Q	R	M	H	A	F	I	T	S	L	S	S	V	G	I	V	S	D	P	D	S	T	D	A	S	602926		
446	E	E	-	-	-	K	K	K	-	-	E	S	S	E	T	V	T	R	F	I	I	S	N	Q	M	Y	-	-	-	GI 1763619		
442	A	A	F	V	S	K	K	K	A	A	E	A	R	W	A	A	Q	E	S	G	I	E	L	D	D	N	Y	R	D	E	GI 116443	
472	S	I	E	D	N	E	D	I	C	N	T	T	S	L	E	N	C	T	A	K	602926											
468	T	I	F	S	M	K	F	A	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	GI 1763619		
472	D	I	F	E	L	Q	H	H	L	L	R	C	L	E	K	-	T	T	M	GI 116443												

FIGURE 4C

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1	M	G	S	G	H	C	L	R	S	T	R	G	S	K	M	V	S	W	S	V	I	A	K	I	Q	E	I	L	Q	R	922119
1	M	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	V	L	E	N	I	Q	S	V	L	Q	K	GI 2350843
31	K	M	V	R	E	F	L	A	E	F	M	S	T	Y	V	M	M	V	F	G	L	G	S	V	A	H	M	V	L	N	922119
16	T	W	V	R	E	F	L	A	E	F	L	N	T	Y	V	L	M	V	F	G	L	G	S	V	A	H	M	V	L	G	GI 2350843
61	K	K	Y	G	S	Y	L	G	V	N	L	G	F	G	F	G	V	T	M	G	V	H	V	A	G	R	I	S	G	A	922119
46	E	R	L	G	S	Y	L	G	V	N	L	G	F	G	F	G	V	T	M	G	I	H	V	A	G	I	S	G	A	GI 2350843	
91	H	M	N	A	A	V	T	F	A	N	C	A	L	G	R	V	P	W	R	K	F	P	V	Y	V	L	G	Q	F	L	922119
76	H	M	N	P	A	V	T	F	T	N	C	A	L	G	R	M	A	G	R	K	F	P	I	Y	V	L	G	Q	F	L	GI 2350843
121	G	S	F	L	A	A	A	T	I	Y	S	L	F	Y	T	A	I	L	H	F	S	G	G	Q	L	M	V	T	G	P	922119
106	G	S	F	L	A	A	A	T	T	Y	L	I	F	Y	G	A	I	N	H	Y	A	G	E	T	L	L	V	T	G	P	GI 2350843
151	V	A	T	A	G	I	F	A	T	Y	L	P	D	H	M	T	L	W	R	G	F	L	N	E	A	W	L	T	G	M	922119
136	K	S	T	A	N	I	F	A	T	Y	L	P	E	H	M	T	L	W	R	G	F	V	D	E	V	F	V	T	G	M	GI 2350843
181	L	Q	L	C	L	F	A	I	T	D	Q	E	N	N	P	A	L	P	G	T	E	A	L	V	I	G	I	L	V	V	922119
166	L	Q	L	C	I	F	A	I	T	D	K	L	N	S	P	A	L	Q	G	T	E	P	L	M	I	G	I	L	V	C	GI 2350843
211	I	I	G	V	S	L	G	M	N	T	G	Y	A	I	N	P	S	R	D	L	P	P	R	I	F	T	F	I	A	G	922119
196	V	L	G	V	S	L	G	M	N	T	G	Y	A	I	N	P	S	R	D	L	P	P	R	F	F	T	F	I	A	G	GI 2350843

FIGURE 5A

241	W	G	K	Q	V	F	S	N	G	E	N	W	W	V	P	V	A	P	L	L	G	A	Y	L	G	G	I	I	922119		
226	W	G	K	K	V	F	S	A	G	N	N	W	W	V	P	V	A	P	L	L	G	A	Y	L	G	G	I	V	GI 2350843		
271	Y	L	V	F	I	G	S	T	I	P	R	E	P	L	K	L	E	D	S	V	A	Y	E	D	H	G	I	T	V	L	922119
256	Y	L	G	L	I	H	A	G	I	P	P	Q	-----														GI 2350843				
301	P	K	M	G	S	H	E	P	T	I	S	P	L	T	P	V	S	V	S	P	A	N	R	S	S	V	H	P	A	P	922119
268	-	-	-	-	G	S															GI 2350843										
331	P	L	H	E	S	M	A	L	E	H	F															922119					
269															GI 2350843																

FIGURE 5B

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1	M N W A F L Q G L L S G V N K Y S T V L S R I W L S V V F I	2731369
1	M N W G F L Q G I L S G V N K Y S T A L G R I W L S V V F I	GI 192647
31	F R V L V Y V V A A E E V W D D E Q K D F D C N T K Q P G C	2731369
31	F R V L V Y V V A A E E V W D D D Q K D F I C N T K Q P G C	GI 192647
61	T N V C Y D N Y F P I S N I R L W A L Q L I L V T C P S L L	2731369
61	P N V C Y D E F F P V S H V R L W A L Q L I L V T C P S L L	GI 192647
91	V V M H V A Y R E E R E R K H H L K H G P N A P S L Y D N L	2731369
91	V V M H V A Y R E E R E R K H R L K H G P N A P A L Y S N L	GI 192647
121	S K K R G G L W W T Y L L S L I F K A A V D A G F L Y I F H	2731369
121	S K K R G G L W W T Y L L S L I F K A A V D S G F L Y I F H	GI 192647
151	R L Y K D Y D M P R V V A C S V E P C P H T V D C Y I S R P	2731369
151	C I Y K D Y D M P R V V A C S V T P C P H T V D C Y I A R P	GI 192647
181	T E K K V F T Y F M V T A A I C I L L N L S E V F Y L V G	2731369
181	T E K K V F T Y F M V V T A A I C I L L N L S E V V Y L V G	GI 192647

FIGURE 6A

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211	K	R	C	M	E	I	F	G	P	R	R	P	R	C	R	E	C	L	P	D	T	C	P	P	Y	V	L	S	2731369	
211	K	R	C	M	E	V	F	R	P	R	R	K	A	S	R	R	H	Q	L	P	D	T	C	P	P	Y	V	I	S	GI 192647
241	Q	G	G	H	P	E	D	G	N	S	V	L	M	K	A	G	S	A	P	V	D	A	G	G	Y	P	2731369			
241	K	G	G	H	P	Q	D	E	S	V	I	L	T	K	A	G	M	A	T	V	D	A	G	V	Y	P	GI 192647			

FIGURE 6B

[illegible]

FIGURE 7

13/16

1	M	A	E	N	H	C	E	L	L	S	P	A	R	G	G	I	G	A	G	L	G	G	L	C	R	R	C	S	A	2243917	
1	M	S	T	V	F	I	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	R	K	S	P	N	g3292929		
31	G	L	G	A	L	A	Q	R	P	G	S	V	S	K	W	V	R	L	N	V	G	G	T	Y	F	L	T	T	R	Q	2243917
14	V	L	-	-	-	-	K	K	Q	G	T	D	Q	W	V	K	L	N	V	G	G	T	Y	F	L	T	T	K	T	g3292929	
61	T	L	C	R	D	P	K	S	F	L	Y	R	L	C	Q	A	D	P	D	L	D	S	D	K	D	E	T	G	A	Y	2243917
39	T	L	S	R	D	P	N	S	F	L	S	R	L	I	Q	E	D	C	D	L	I	S	D	R	D	E	T	G	A	Y	g3292929
91	L	I	D	R	D	P	T	Y	F	G	P	V	L	N	Y	L	R	H	G	K	L	V	I	N	K	D	L	A	E	E	2243917
69	L	I	D	R	D	P	K	Y	F	A	P	V	L	N	Y	L	R	H	G	K	L	V	L	D	-	G	V	S	E	E	g3292929
121	G	V	L	E	E	A	E	F	Y	N	I	T	S	L	I	K	L	V	K	D	K	I	R	E	R	D	S	K	T	S	2243917
98	G	V	L	E	E	A	E	F	Y	N	V	T	Q	L	I	A	L	K	E	C	I	L	H	R	D	Q	R	-	P	g3292929	
151	Q	V	P	V	K	H	V	Y	R	V	L	Q	C	Q	E	E	E	L	T	Q	M	V	S	T	M	S	D	G	W	K	2243917
127	Q	T	D	K	K	R	V	Y	R	V	L	Q	C	R	E	Q	E	L	T	Q	M	I	S	T	L	S	D	G	W	R	g3292929
181	F	E	Q	L	V	S	I	G	S	S	Y	N	Y	G	N	E	D	Q	A	E	F	L	C	V	V	S	K	E	L	H	2243917
157	F	E	Q	L	I	S	M	Q	Y	T	-	N	Y	G	P	F	E	N	N	E	F	L	C	V	V	S	K	E	-	C	g3292929
211	N	T	P	Y	G	T	A	S	E	P	S	E	K	A	K	I	L	Q	E	R	G	S	R	-	-	-	-	-	-	-	2243917
185	G	T	A	G	R	E	L	E	L	N	D	R	A	K	V	L	Q	Q	K	G	S	R	I	N	T	I	S	H	S	g3292929	

FIGURE 8A

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FIGURE 8B



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1 MVF - - TQAPAEIMGHLRIRSLARQCLAEF 2597476  
 1 MQPEGAEGKSFKQRLVLKSSLAKE TLSEF g2887407  
 29 LGVFLMLLTQGA V AQA V TSGETKGNFFTM 2597476  
 31 LGTFILIVLGCVCVAQA ILSRGRFGGVITI g2887407  
 59 FLAGSLAVTIAIYVGGNVSGAHLP AFSLA 2597476  
 61 NVGFSMAVAMAIYVAGVSGGHI NPAVSLA g2887407  
 89 MCIVGRLPWVKLP IYILVQLLSAF C ASGAT 2597476  
 91 MCLFGRMKWF KLPFYVGAQLGAFVGAATV g2887407  
 119 YVLYHDA LQNYTGGNLTVTGPKETASIFAT 2597476  
 121 FGIY YDGLMSFAGGKLLIVGENATAHIFAT g2887407  
 149 YPAPYLSLNNGFLDQVLGTGMLIVGLLAIL 2597476  
 151 YPAPYLSLANAFADQV VATMILLIIVFAIF g2887407  
 179 DR RNKGVPAGLEPVVVGMLILALGLSMGAN 2597476  
 181 DSRNLGAPRGLEPIAIGLLIIVIASLGLN g2887407  
 209 CGIPLNPARDLGPRLFTYVAGWGP EVFSAG 2597476  
 211 SGCAMNPARDLS PRLFTALAGWGF EVFRAG g2887407

FIGURE 9A

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239 N G W W V P V V A P L V G A T V G T A T Y Q L L V A L H H  
 241 N N F W W I P V V G P L V G A V I G G L I Y V L V I E I H H

2597476  
 92887407

269 P E G P E P A Q D L V S A Q H K A S E L E T P A S A Q M L E  
 271 P E - P D S V F K A E Q S E D K P E K Y E - - - - - L S

2597476  
 92887407

299 C K L  
 293 V I M

2597476  
 92887407

Docket No.: PF-0589 USN

**DECLARATION AND POWER OF ATTORNEY FOR  
UNITED STATES PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,  
and

I believe that I am the original, first and sole inventor (if only one name is listed below)  
or an original, first and joint inventor (if more than one name is listed below) of the subject  
matter which is claimed and for which a United States patent is sought on the invention entitled

**HUMAN MEMBRANE CHANNEL PROTEINS**

the specification of which:

   /    / is attached hereto.

  X   / was filed on February 27, 2001, as application Serial No. 09/786,133 and if this box  
contains an X    /, was amended on \_\_\_\_\_.

  X   / was filed as Patent Cooperation Treaty international application No. PCT/US99/20468  
on 02 September 1999, if this box contains an X    /, was amended on under Patent Cooperation  
Treaty Article 19 on \_\_\_\_\_ 2001, and if this box contains an X    /, was amended on \_\_\_\_\_.

I hereby state that I have reviewed and understand the contents of the above-identified  
specification, including the claims, as amended by any amendment referred to above.

I acknowledge my duty to disclose information which is material to the examination of  
this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim the benefit under Title 35, United States Code, §119 or §365(a)-(b) of any  
foreign application(s) for patent or inventor's certificate indicated below and of any Patent  
Cooperation Treaty international applications(s) designating at least one country other than the  
United States indicated below and have also identified below any foreign application(s) for  
patent or inventor's certificate and Patent Cooperation Treaty international application(s)  
designating at least one country other than the United States for the same subject matter and  
having a filing date before that of the application for said subject matter the priority of which is  
claimed:

**Docket No.: PF-0589 USN**

Country	Number	Filing Date	Priority Claimed
_____	_____	_____	// Yes // No
_____	_____	_____	// Yes // No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)
60/240,932	November 12, 1998	Expired
60/155,225	December 9, 1998	Expired
60/155,216	January 26, 1999	Expired
60/155,263	February 10, 1999	Expired
60/155,226	September 2, 1998	Expired

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of Title 35, United States Code §112, I acknowledge my duty to disclose material information as defined in Title 37 Code of Federal Regulations, §1.56(a) which occurred between the filing date(s) of the prior application(s) and the national or Patent Cooperation Treaty international filing date of this application:

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)
_____	_____	_____

I hereby appoint the following:

Lucy J. Billings	Reg. No. <u>36,749</u>
Michael C. Cerrone	Reg. No. <u>39,132</u>
Diana Hamlet-Cox	Reg. No. <u>33,302</u>
Richard C. Ekstrom	Reg. No. <u>37,027</u>
Barrie D. Greene	Reg. No. <u>46,740</u>
Matthew R. Kaser	Reg. No. <u>44,817</u>
Lynn E. Murry	Reg. No. <u>42,918</u>
Shirley A. Recipon	Reg. No. <u>47,016</u>
Susan K. Sather	Reg. No. <u>44,316</u>
Michelle M. Stempien	Reg. No. <u>41,327</u>
David G. Streeter	Reg. No. <u>43,168</u>
Stephen Todd	Reg. No. <u>47,139</u>
Christopher Turner	Reg. No. <u>45,167</u>
P. Ben Wang	Reg. No. <u>41,420</u>

respectively and individually, as my patent attorneys and/or agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Please address all communications to:

Docket No.: PF-0589 USN

**LEGAL DEPARTMENT**  
**INCYTE GENOMICS, INC.**  
3160 PORTER DRIVE, PALO ALTO, CA 94304

TEL: 650-855-0555      FAX: 650-849-8886 or 650-845-4166

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

**Sole Inventor or  
First Joint Inventor:**

100

**Full name:** JANICE AU-YOUNG  
**Signature:** *Janice Au-Young*  
**Date:** February 2, 2001  
**Citizenship** United States  
**Residence:** Brisbane, California CA  
**P.O. Address:** 233 Golden Eagle Lane  
Brisbane, California 94005

**Second Joint Inventor:**

200

**Full name:** OLGA BANDMAN  
**Signature:** *Olga Bandman*  
**Date:** 16 February, 2001  
**Citizenship** United States CA  
**Residence:** Mountain View, California  
**P.O. Address:** 366 Anna Avenue  
Mountain View, California 94005

Docket No.: PF-0589 USN

## Third Joint Inventor:

300

Full name:

Y. TOM TANG

Signature:

Y. Tom Tang

Date:

February 27, 2001

Citizenship

People's Republic of China USA u.s. 100

Residence:

San Jose, California CA

P.O. Address:

4230 Ranwick Court  
San Jose, California 95118

## Fourth Joint Inventor:

400

Full name:

ROOPA REDDY

Signature:

Roopa Reddy

Date:

March 1st, 2001

Citizenship

India

Residence:

Sunnyvale, California CA

P.O. Address:

1233 W. McKinley Drive  
Sunnyvale, California 94086

## Fifth Joint Inventor:

500

Full name:

JENNIFER L. HILLMAN

Signature:

Jill Hillman

Date:

February 16, 2001

Citizenship

United States CA

Residence:

Mountain View, California

P.O. Address:

230 Monroe Drive  
#12  
Mountain View, California 94040

Docket No.: PF-0589 USN

## Sixth Joint Inventor:

600

Full name:

HENRY YUE

Signature:

Henry Yue

Date:

March 02, 2001

Citizenship

United States

Residence:

Sunnyvale, California CA

P.O. Address:

826 Lois Avenue  
Sunnyvale, California 94087

## Seventh Joint Inventor:

200

Full name:

PREETI LAL

Signature:

Preeti Lal

Date:

FEBRUARY, 16, 2001

Citizenship

United States CA

Residence:

Santa Clara, California

P.O. Address:

P.O. Box 5142  
Santa Clara, California 95056

## Eighth Joint Inventor:

800

Full name:

NEIL C. CORLEY

Signature:

Neil C. Corley

Date:

MARCH 5, 2001

Citizenship

United States CA

Residence:

Castro Valley, California

P.O. Address:

20426 Crow Creek Road  
Castro Valley, California 94552

Docket No.: PF-0589 USN

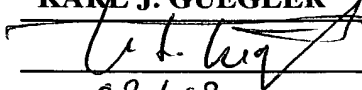
## Ninth Joint Inventor:

900

Full name:

KARL J. GUEGLER

Signature:



Date:

02/02, 2001

Citizenship

United States

Residence:

Menlo Park, California *CA*

P.O. Address:

1048 Oakland Avenue  
Menlo Park, California 94025

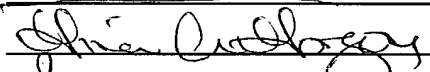
## Tenth Joint Inventor:

1000

Full name:

GINA A. GORGONE

Signature:



Date:

Feb 5, 2001

Citizenship

United States

Residence:

Boulder Creek, California *CA*

P.O. Address:

1253 Pinecrest Drive  
Boulder Creek, California 95006

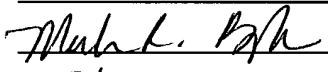
## Eleventh Joint Inventor:

1100

Full name:

MARIAH R. BAUGHN

Signature:



Date:

February 12, 2001

Citizenship

United States

Residence:

San Leandro, California *CA*

P.O. Address:

14244 Santiago Road  
San Leandro, California 94577



Docket No.: PF-0589 USN

Twelfth Joint Inventor:

1200

Full name:

YALDA AZIMZAI

Signature:

*Yalda Azimzai*

Date:

February 28, 2001

Citizenship

United States *et*

Residence:

Castro Valley, California

P.O. Address:

5518 Boulder Canyon Drive  
Castro Valley, California 94552



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	95		100		105
Gln Ala Ser Val	Ile Ala Glu Arg Phe	Val Ser Ser Phe Ser	Arg		
	110		115		120
Arg Ser Ser Val	Ala Gln Glu Asp Ser	Lys Ser Ser Gly Phe	Gly		
	125		130		135
Ser Pro Arg Leu	Val Ser Arg Ser Ser	Ser Val Leu Ser Leu	Glu		
	140		145		150
Gly Ser Glu Lys	Gly Leu Ala Arg His	Gly Ser Ala Thr Asp	Ser		
	155		160		165
Leu Ser Cys Gln	Leu Ser Pro Glu Val	Asp Ile Ser Val Gly	Val		
	170		175		180
Ala Thr Glu Asp	Ser Pro Ser Val Asn	Gly Met Glu Pro Pro	Ser		
	185		190		195
Pro Gly Cys Pro	Val Glu Pro Asp Arg	Ser Ser Cys Lys Lys	Lys		
	200		205		210
Glu Ser Ala Leu	Ser Thr Arg Asp Arg	Leu Leu Leu Asp Lys	Ile		
	215		220		225
Lys Ser Tyr Tyr	Glu Asn Ala Glu His	His Asp Ala Gly Phe	Ser		
	230		235		240
Val Arg Arg Arg	Glu Ser Leu Ser Tyr	Ile Pro Lys Gly Leu	Val		
	245		250		255
Arg Asn Ser Ile	Ser Arg Phe Asn Ser	Leu Pro Arg Pro Asp	Pro		
	260		265		270
Glu Pro Val Pro	Pro Val Gly Ser Lys	Arg Gln Val Gly Ser	Arg		
	275		280		285
Pro Thr Ser Trp	Ala Leu Phe Glu Leu	Pro Gly Pro Ser Gln	Ala		
	290		295		300
Val Lys Gly Asp	Pro Pro Pro Ile Ser	Asp Ala Glu Phe Arg	Pro		
	305		310		315
Ser Ser Glu Ile	Val Lys Ile Trp Glu	Gly Met Glu Ser Ser	Gly		
	320		325		330
Gly Ser Pro Gly	Lys Gly Pro Gly Gln	Gly Gln Ala Asn Gly	Phe		
	335		340		345
Asp Leu His Glu	Pro Leu Phe Ile Leu	Glu Glu His Glu Leu	Gly		
	350		355		360
Ala Ile Thr Glu	Glu Ser Ala Thr Ala	Ser Pro Glu Ser Ser	Ser		
	365		370		375
Pro Thr Glu Gly	Arg Ser Pro Ala His	Leu Ala Arg Glu Leu	Lys		
	380		385		390
Glu Leu Val Lys	Glu Leu Ser Ser Ser	Thr Gln Gly Glu Leu	Val		
	395		400		405
Ala Pro Leu His	Pro Arg Ile Val Gln	Leu Ser His Val Met	Asp		
	410		415		420
Ser His Val Ser	Glu Arg Val Lys Asn	Lys Val Tyr Gln Leu	Ala		
	425		430		435
Arg Gln Tyr Ser	Leu Arg Ile Lys Ser	Asn Lys Pro Val Met	Ala		
	440		445		450
Arg Pro Pro Leu	Gln Trp Glu Lys Val	Ala Pro Glu Arg Asp	Gly		
	455		460		465
Lys Ser Pro Thr	Val Pro Cys Leu Gln	Glu Glu Ala Gly Glu	Pro		
	470		475		480
Leu Gly Gly Lys	Gly Lys Arg Lys Pro	Val Leu Ser Leu Phe	Asp		
	485		490		495
Tyr Glu Gln Leu	Met Ala Gln Glu His	Ser Pro Pro Lys Pro	Ser		
	500		505		510
Ser Ala Gly Glu	Met Ser Pro Gln Arg	Phe Phe Phe Asn Pro	Pro		

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515	520	525
Ala Val Ser Gln Arg Thr Thr Ser Pro Gly Gly Arg Pro Ser Ala		
530	535	540
Arg Ser Pro Leu Ser Pro Thr Glu Thr Phe Ser Trp Pro Asp Val		
545	550	555
Arg Glu Leu Cys Ser Lys Tyr Ala Ser Arg Asp Glu Ala Arg Arg		
560	565	570
Ala Gly Gly Gly Arg Pro Arg Gly Pro Pro Val Asn Arg Ser His		
575	580	585
Ser Val Pro Glu Asn Met Val Glu Pro Pro Leu Ser Gly Arg Val		
590	595	600
Gly Arg Cys Arg Ser Leu Ser Thr Lys Arg Gly Arg Gly Gly Gly		
605	610	615
Glu Ala Ala Gln Ser Pro Gly Pro Leu Pro Gln Ser Lys Pro Asp		
620	625	630
Gly Gly Glu Thr Leu Tyr Val Thr Ala Asp Leu Thr Leu Glu Asp		
635	640	645
Asn Arg Arg Val Ile Val Met Glu Lys Gly Pro Leu Pro Ser Pro		
650	655	660
Thr Ala Gly Leu Glu Glu Ser Ser Gly Gln Gly Pro Ser Ser Pro		
665	670	675
Val Ala Leu Leu Gly Gln Val Gln Asp Phe Gln Gln Ser Ala Glu		
680	685	690
Cys Gln Pro Lys Glu Glu Gly Ser Arg Asp Pro Ala Asp Pro Ser		
695	700	705
Gln Gln Gly Arg Val Arg Asn Leu Arg Glu Lys Phe Gln Ala Leu		
710	715	720
Asn Ser Val Gly		

<210> 2

<211> 257

<212> PRT

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 4094907CD1

<400> 2

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Asn Gln Gly Ile Pro Thr Pro Ala Gln Leu Thr Lys Ser Asn Ala		15
20	25	30
Pro Val His Ile Asp Val Gly Gly His Met Tyr Thr Ser Ser Leu		
35	40	45
Ala Thr Leu Thr Lys Tyr Pro Glu Ser Arg Ile Gly Arg Leu Phe		
50	55	60
Asp Gly Thr Glu Pro Ile Val Leu Asp Ser Leu Lys Gln His Tyr		
65	70	75
Phe Ile Asp Arg Asp Gly Gln Met Phe Arg Tyr Ile Leu Asn Phe		
80	85	90
Leu Arg Thr Ser Lys Leu Leu Ile Pro Asp Asp Phe Lys Asp Tyr		
95	100	105
Thr Leu Leu Tyr Glu Glu Ala Lys Tyr Phe Gln Leu Gln Pro Met		
110	115	120

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Leu	Leu	Glu	Met	Glu	Arg	Trp	Lys	Gln	Asp	Arg	Glu	Thr	Gly	Arg
				125					130					135
Phe	Ser	Arg	Pro	Cys	Glu	Cys	Leu	Val	Val	Arg	Val	Ala	Pro	Asp
				140					145					150
Leu	Gly	Glu	Arg	Ile	Thr	Leu	Ser	Gly	Asp	Lys	Ser	Leu	Ile	Glu
				155					160					165
Glu	Val	Phe	Pro	Glu	Ile	Gly	Asp	Val	Met	Cys	Asn	Ser	Val	Asn
				170					175					180
Ala	Gly	Trp	Asn	His	Asp	Ser	Thr	His	Val	Ile	Arg	Phe	Pro	Leu
				185					190					195
Asn	Gly	Tyr	Cys	His	Leu	Asn	Ser	Val	Gln	Val	Leu	Glu	Arg	Leu
				200					205					210
Gln	Gln	Arg	Gly	Phe	Glu	Ile	Val	Gly	Ser	Cys	Gly	Gly	Gly	Val
				215					220					225
Asp	Ser	Ser	Gln	Phe	Ser	Glu	Tyr	Val	Leu	Arg	Arg	Glu	Leu	Arg
				230					235					240
Arg	Thr	Pro	Arg	Val	Pro	Ser	Val	Ile	Arg	Ile	Lys	Gln	Glu	Pro
				245					250					255

Leu Asp

<210> 3

<211> 377

<212> PRT

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 518158CD1

<400> 3

Met	Gly	Gly	Asp	Leu	Val	Leu	Gly	Leu	Gly	Ala	Leu	Arg	Arg	Arg
1				5					10					15
Lys	Arg	Leu	Leu	Glu	Gln	Glu	Lys	Ser	Leu	Ala	Gly	Trp	Ala	Leu
				20					25					30
Val	Leu	Ala	Gly	Thr	Gly	Ile	Gly	Leu	Met	Val	Leu	His	Ala	Glu
				35					40					45
Met	Leu	Trp	Phe	Gly	Gly	Cys	Ser	Trp	Ala	Leu	Tyr	Leu	Phe	Leu
				50					55					60
Val	Lys	Cys	Thr	Ile	Ser	Ile	Ser	Thr	Phe	Leu	Leu	Leu	Cys	Leu
				65					70					75
Ile	Val	Ala	Phe	His	Ala	Lys	Glu	Val	Gln	Leu	Phe	Met	Thr	Asp
				80					85					90
Asn	Gly	Leu	Arg	Asp	Trp	Arg	Val	Ala	Leu	Thr	Gly	Arg	Gln	Ala
				95					100					105
Ala	Gln	Ile	Val	Leu	Glu	Leu	Val	Val	Cys	Gly	Leu	His	Pro	Ala
				110					115					120
Pro	Val	Arg	Gly	Pro	Pro	Cys	Val	Gln	Asp	Leu	Gly	Ala	Pro	Leu
				125					130					135
Thr	Ser	Pro	Gln	Pro	Trp	Pro	Gly	Phe	Leu	Gly	Gln	Gly	Glu	Ala
				140					145					150
Leu	Leu	Ser	Leu	Ala	Met	Leu	Leu	Leu	Gly	Leu	Thr	Leu	Gly	Leu
				155					160					165
Trp	Leu	Thr	Thr	Ala	Trp	Val	Leu	Ser	Val	Ala	Glu	Arg	Gln	Ala
				170					175					180
Val	Asn	Ala	Thr	Gly	His	Leu	Ser	Asp	Thr	Leu	Trp	Leu	Ile	Pro

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	185		190		195
Ile Thr Phe Leu	Thr Ile Gly Tyr Gly	Asp Val Val Pro Gly	Thr		
	200		205		210
Met Trp Gly Lys	Ile Val Cys Leu Cys	Thr Gly Val Met Gly	Val		
	215		220		225
Cys Cys Thr Ala	Leu Leu Val Ala Val	Val Ala Arg Lys Leu	Glu		
	230		235		240
Phe Asn Lys Ala	Glu Lys His Val His	Asn Phe Met Met Asp	Ile		
	245		250		255
Gln Tyr Thr Lys	Glu Met Lys Glu Ser	Ala Ala Arg Val Leu	Gln		
	260		265		270
Glu Ala Trp Met	Phe Tyr Lys His Thr	Arg Arg Lys Glu Ser	His		
	275		280		285
Ala Ala Arg Arg	His Gln Arg Lys Leu	Leu Ala Ala Ile Asn	Ala		
	290		295		300
Phe Arg Gln Val	Arg Leu Lys His Arg	Lys Leu Arg Glu Gln	Val		
	305		310		315
Asn Ser Met Val	Asp Ile Ser Lys Met	His Met Ile Leu Tyr	Asp		
	320		325		330
Leu Gln Gln Asn	Leu Ser Ser Ser His	Arg Ala Leu Glu Lys	Gln		
	335		340		345
Ile Asp Thr Leu	Ala Gly Lys Leu Asp	Ala Leu Thr Glu Leu	Leu		
	350		355		360
Ser Thr Ala Leu	Gly Pro Arg Gln Leu	Pro Glu Pro Ser Gln	Gln		
	365		370		375
Ser Lys					

<210> 4  
 <211> 491  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 602926CD1

<400> 4	
Met Val Phe Gly Glu Phe Phe His Arg Pro Gly Gln Asp Glu Glu	
1	5 10 15
Leu Val Asn Leu Asn Val Gly Gly Phe Lys Gln Ser Val Asp Gln	
	20 25 30
Ser Thr Leu Leu Arg Phe Pro His Thr Arg Leu Gly Lys Leu Leu	
	35 40 45
Thr Cys His Ser Glu Glu Ala Ile Leu Glu Leu Cys Asp Asp Tyr	
	50 55 60
Ser Val Ala Asp Lys Glu Tyr Tyr Phe Asp Arg Asn Pro Ser Ser	
	65 70 75
Phe Arg Tyr Val Leu Asn Phe Tyr Tyr Thr Gly Lys Leu His Val	
	80 85 90
Met Glu Glu Leu Cys Val Phe Ser Phe Cys Gln Glu Ile Glu Tyr	
	95 100 105
Trp Gly Ile Asn Glu Leu Phe Ile Asp Ser Cys Cys Ser Asn Arg	
	110 115 120
Tyr Gln Glu Arg Lys Glu Glu Asn His Glu Lys Asp Trp Asp Gln	
	125 130 135

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Lys Ser His Asp Val Ser Thr Asp Ser Ser Phe Glu Glu Ser Ser	140	145	150
Leu Phe Glu Lys Glu Leu Glu Lys Phe Asp Thr Leu Arg Phe Gly	155	160	165
Gln Leu Arg Lys Lys Ile Trp Ile Arg Met Glu Asn Pro Ala Tyr	170	175	180
Cys Leu Ser Ala Lys Leu Ile Ala Ile Ser Ser Leu Ser Val Val	185	190	195
Leu Ala Ser Ile Val Ala Met Cys Val His Ser Met Ser Glu Phe	200	205	210
Gln Asn Glu Asp Gly Glu Val Asp Asp Pro Val Leu Glu Gly Val	215	220	225
Glu Ile Ala Cys Ile Ala Trp Phe Thr Gly Glu Leu Ala Val Arg	230	235	240
Leu Ala Ala Ala Pro Cys Gln Lys Lys Phe Trp Lys Asn Pro Leu	245	250	255
Asn Ile Ile Asp Phe Val Ser Ile Ile Pro Phe Tyr Ala Thr Leu	260	265	270
Ala Val Asp Thr Lys Glu Glu Glu Ser Glu Asp Ile Glu Asn Met	275	280	285
Gly Lys Val Val Gln Ile Leu Arg Leu Met Arg Ile Phe Arg Ile	290	295	300
Leu Lys Leu Ala Arg His Ser Val Gly Leu Arg Ser Leu Gly Ala	305	310	315
Thr Leu Arg His Ser Tyr His Glu Val Gly Leu Leu Leu Leu Phe	320	325	330
Leu Ser Val Gly Ile Ser Ile Phe Ser Val Leu Ile Tyr Ser Val	335	340	345
Glu Lys Asp Asp His Thr Ser Ser Leu Thr Ser Ile Pro Ile Cys	350	355	360
Trp Trp Trp Ala Thr Ile Ser Met Thr Thr Val Gly Tyr Gly Asp	365	370	375
Thr His Pro Val Thr Leu Ala Gly Lys Leu Ile Ala Ser Thr Cys	380	385	390
Ile Ile Cys Gly Ile Leu Val Val Ala Leu Pro Ile Thr Ile Ile	395	400	405
Phe Asn Lys Phe Ser Lys Tyr Tyr Gln Lys Gln Lys Asp Ile Asp	410	415	420
Val Asp Gln Cys Ser Glu Asp Ala Pro Glu Lys Cys His Glu Leu	425	430	435
Pro Tyr Phe Asn Ile Arg Asp Ile Tyr Ala Gln Arg Met His Ala	440	445	450
Phe Ile Thr Ser Leu Ser Ser Val Gly Ile Val Val Ser Asp Pro	455	460	465
Asp Ser Thr Asp Ala Ser Ser Ile Glu Asp Asn Glu Asp Ile Cys	470	475	480
Asn Thr Thr Ser Leu Glu Asn Cys Thr Ala Lys	485	490	

<210> 5

<211> 341

<212> PRT

<213> Homo sapiens

<220>





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<220>  
<221> misc\_feature  
<223> Incyte ID No: 2666782CD1

<400> 6

Met	Gly	Ile	Lys	Phe	Leu	Glu	Val	Ile	Lys	Pro	Phe	Cys	Ala	Val	1	5	10	15
Leu	Pro	Glu	Ile	Gln	Lys	Pro	Glu	Arg	Lys	Ile	Gln	Phe	Arg	Glu	20	25	30	
Lys	Val	Leu	Trp	Thr	Ala	Ile	Thr	Leu	Phe	Ile	Phe	Leu	Val	Cys	35	40	45	
Cys	Gln	Ile	Pro	Leu	Phe	Gly	Ile	Met	Ser	Ser	Asp	Ser	Ala	Asp	50	55	60	
Pro	Phe	Tyr	Trp	Met	Arg	Val	Ile	Leu	Ala	Ser	Asn	Arg	Gly	Thr	65	70	75	
Leu	Met	Glu	Leu	Gly	Ile	Ser	Pro	Ile	Val	Thr	Ser	Gly	Leu	Ile	80	85	90	
Met	Gln	Leu	Leu	Ala	Gly	Ala	Lys	Ile	Ile	Glu	Val	Gly	Asp	Thr	95	100	105	
Pro	Lys	Asp	Arg	Ala	Leu	Phe	Asn	Gly	Ala	Gln	Lys	Leu	Phe	Gly	110	115	120	
Met	Ile	Ile	Thr	Ile	Gly	Gln	Ala	Ile	Val	Tyr	Val	Met	Thr	Gly	125	130	135	
Met	Tyr	Gly	Asp	Pro	Ala	Glu	Met	Gly	Ala	Gly	Ile	Cys	Leu	Leu	140	145	150	
Ile	Ile	Ile	Gln	Leu	Phe	Val	Thr	Ser	Leu	Ile	Val	Leu	Leu	Leu	155	160	165	
Asp	Glu	Leu	Leu	Gln	Thr	Gly	Tyr	Ser	Leu	Gly	Ser	Gly	Ile	Ser	170	175	180	
Leu	Val	Ile	Ala	Thr	Asn	Ile	Cys	Glu	Thr	Ile	Val	Trp	Lys	Ala	185	190	195	
Phe	Ser	Pro	Thr	Thr	Ile	Asn	Thr	Gly	Arg	Gly	Thr	Glu	Phe	Glu	200	205	210	
Gly	Ala	Val	Ile	Ala	Leu	Phe	His	Leu	Leu	Ala	Thr	Arg	Thr	Asp	215	220	225	
Lys	Val	Arg	Ala	Leu	Arg	Glu	Ala	Phe	Tyr	Arg	Gln	Asn	Leu	Pro	230	235	240	
Asn	Leu	Met	Asn	Leu	Ile	Ala	Thr	Val	Phe	Val	Phe	Ala	Val	Val	245	250	255	
Ile	Tyr	Phe	Gln	Gly	Phe	Arg	Val	Asp	Leu	Pro	Ile	Lys	Ser	Ala	260	265	270	
Arg	Tyr	Arg	Gly	Gln	Tyr	Ser	Ser	Tyr	Pro	Ile	Lys	Leu	Phe	Tyr	275	280	285	
Thr	Ser	Asn	Ile	Pro	Ile	Ile	Leu	Gln	Ser	Ala	Leu	Val	Ser	Asn	290	295	300	
Leu	Tyr	Val	Ile	Ser	Gln	Met	Leu	Ser	Val	Arg	Phe	Ser	Gly	Asn	305	310	315	
Phe	Leu	Val	Asn	Leu	Leu	Gly	Gln	Trp	Ala	Asp	Val	Ser	Gly	Gly	320	325	330	
Gly	Pro	Ala	Arg	Ser	Tyr	Pro	Val	Gly	Gly	Leu	Cys	Tyr	Tyr	Leu	335	340	345	
Ser	Pro	Pro	Glu	Ser	Met	Gly	Ala	Ile	Phe	Glu	Asp	Pro	Val	His	350	355	360	
Val	Val	Val	Tyr	Ile	Ile	Phe	Met	Leu	Gly	Ser	Cys	Ala	Phe	Phe	365	370	375	
Ser	Lys	Thr	Trp	Ile	Glu	Val	Ser	Gly	Ser	Ser	Ala	Lys	Asp	Val				

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380          385          390
Ala Lys Gln Leu Lys Glu Gln Gln Met Val Met Arg Gly His Arg
395          400          405
Asp Thr Ser Met Val His Glu Leu Asn Arg Tyr Ile Pro Thr Ala
410          415          420
Ala Ala Phe Gly Gly Leu Cys Ile Gly Ala Leu Ser Val Leu Ala
425          430          435
Asp Phe Leu Gly Ala Ile Gly Ser Gly Thr Gly Ile Leu Leu Ala
440          445          450
Val Thr Ile Ile Tyr Gln Tyr Phe Glu Ile Phe Val Lys Glu Gln
455          460          465
Ala Glu Val Gly Gly Met Gly Ala Leu Phe Phe
470          475

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<210> 7
<211> 266
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 2731369CD1

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<400> 7
Met Asn Trp Ala Phe Leu Gln Gly Leu Leu Ser Gly Val Asn Lys
 1          5          10          15
Tyr Ser Thr Val Leu Ser Arg Ile Trp Leu Ser Val Val Phe Ile
20          25          30
Phe Arg Val Leu Val Tyr Val Val Ala Ala Glu Glu Val Trp Asp
35          40          45
Asp Glu Gln Lys Asp Phe Asp Cys Asn Thr Lys Gln Pro Gly Cys
50          55          60
Thr Asn Val Cys Tyr Asp Asn Tyr Phe Pro Ile Ser Asn Ile Arg
65          70          75
Leu Trp Ala Leu Gln Leu Ile Leu Val Thr Cys Pro Ser Leu Leu
80          85          90
Val Val Met His Val Ala Tyr Arg Glu Glu Arg Glu Arg Lys His
95          100          105
His Leu Lys His Gly Pro Asn Ala Pro Ser Leu Tyr Asp Asn Leu
110          115          120
Ser Lys Lys Arg Gly Gly Leu Trp Trp Thr Tyr Leu Leu Ser Leu
125          130          135
Ile Phe Lys Ala Ala Val Asp Ala Gly Phe Leu Tyr Ile Phe His
140          145          150
Arg Leu Tyr Lys Asp Tyr Asp Met Pro Arg Val Val Ala Cys Ser
155          160          165
Val Glu Pro Cys Pro His Thr Val Asp Cys Tyr Ile Ser Arg Pro
170          175          180
Thr Glu Lys Lys Val Phe Thr Tyr Phe Met Val Thr Thr Ala Ala
185          190          195
Ile Cys Ile Leu Leu Asn Leu Ser Glu Val Phe Tyr Leu Val Gly
200          205          210
Lys Arg Cys Met Glu Ile Phe Gly Pro Arg His Arg Arg Pro Arg
215          220          225
Cys Arg Glu Cys Leu Pro Asp Thr Cys Pro Pro Tyr Val Leu Ser

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	230		235		240
Gln Gly Gly His	Pro Glu Asp Gly Asn	Ser Val Leu Met Lys	Ala		
	245		250		255
Gly Ser Ala Pro	Val Asp Ala Gly Gly Tyr	Pro			
	260		265		

<210> 8  
 <211> 182  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 1375415CD1

<400> 8

Met Ala Glu Phe	Pro Ser Lys Val Ser Thr Arg Thr Ser Ser Pro
1	5 10 15
Ala Gln Gly Ala Glu	Ala Ser Val Ser Ala Leu Arg Pro Asp Leu
	20 25 30
Gly Phe Val Arg Ser	Arg Leu Gly Ala Leu Met Leu Leu Gln Leu
	35 40 45
Val Leu Gly Leu Leu	Val Trp Ala Leu Ile Ala Asp Thr Pro Tyr
	50 55 60
His Leu Tyr Pro Ala	Tyr Gly Trp Val Met Phe Val Ala Val Phe
	65 70 75
Leu Trp Leu Val Thr	Ile Val Leu Phe Asn Leu Tyr Leu Phe Gln
	80 85 90
Leu His Met Lys Leu	Tyr Met Val Pro Trp Pro Leu Val Leu Met
	95 100 105
Ile Phe Asn Ile Ser	Ala Thr Val Leu Tyr Ile Thr Ala Phe Ile
	110 115 120
Ala Cys Ser Ala Ala	Val Asp Leu Thr Ser Leu Arg Gly Thr Arg
	125 130 135
Pro Tyr Asn Gln Arg	Ala Ala Ala Ser Phe Phe Ala Cys Leu Val
	140 145 150
Met Ile Ala Tyr Gly	Val Ser Ala Phe Phe Ser Tyr Gln Ala Trp
	155 160 165
Arg Gly Val Gly Ser	Asn Ala Ala Thr Ser Gln Met Ala Gly Gly
	170 175 180
Tyr Ala	

<210> 9  
 <211> 942  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 2733282CD1

<400> 9

Met Thr Gln Arg Ser	Ile Ala Gly Pro Ile Cys Asn Leu Lys Phe
1	5 10 15

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Val Thr Leu Leu Val Ala Leu Ser Ser Glu Leu Pro Phe Leu Gly	20	25	30
Ala Gly Val Gln Leu Gln Asp Asn Gly Tyr Asn Gly Leu Leu Ile	35	40	45
Ala Ile Asn Pro Gln Val Pro Glu Asn Gln Asn Leu Ile Ser Asn	50	55	60
Ile Lys Glu Met Ile Thr Glu Ala Ser Phe Tyr Leu Phe Asn Ala	65	70	75
Thr Lys Arg Arg Val Phe Phe Arg Asn Ile Lys Ile Leu Ile Pro	80	85	90
Ala Thr Trp Lys Ala Asn Asn Asn Ser Lys Ile Lys Gln Glu Ser	95	100	105
Tyr Glu Lys Ala Asn Val Ile Val Thr Asp Trp Tyr Gly Ala His	110	115	120
Gly Asp Asp Pro Tyr Thr Leu Gln Tyr Arg Gly Cys Gly Lys Glu	125	130	135
Gly Lys Tyr Ile His Phe Thr Pro Asn Phe Leu Leu Asn Asp Asn	140	145	150
Leu Thr Ala Gly Tyr Gly Ser Arg Gly Arg Val Phe Val His Glu	155	160	165
Trp Ala His Leu Arg Trp Gly Val Phe Asp Glu Tyr Asn Asn Asp	170	175	180
Lys Pro Phe Tyr Ile Asn Gly Gln Asn Gln Ile Lys Val Thr Arg	185	190	195
Cys Ser Ser Asp Ile Thr Gly Ile Phe Val Cys Glu Lys Gly Pro	200	205	210
Cys Pro Gln Glu Asn Cys Ile Ile Ser Lys Leu Phe Lys Glu Gly	215	220	225
Cys Thr Phe Ile Tyr Asn Ser Thr Gln Asn Ala Thr Ala Ser Ile	230	235	240
Met Phe Met Gln Ser Tyr Leu Cys Gly Glu Ile Cys Asn Ala Ser	245	250	255
Thr His Asn Gln Glu Ala Pro Asn Leu Gln Asn Gln Met Cys Ser	260	265	270
Leu Arg Ser Ala Trp Asp Val Ile Thr Asp Ser Ala Asp Phe His	275	280	285
His Ser Phe Pro Met Asn Gly Thr Glu Leu Pro Pro Pro Pro Thr	290	295	300
Phe Ser Leu Val Glu Ala Gly Asp Lys Val Val Cys Leu Val Leu	305	310	315
Asp Val Ser Ser Lys Met Ala Glu Ala Asp Arg Leu Leu Gln Leu	320	325	330
Gln Gln Ala Ala Glu Phe Tyr Leu Met Gln Ile Val Glu Ile His	335	340	345
Thr Phe Val Gly Ile Ala Ser Phe Asp Ser Lys Gly Glu Ile Arg	350	355	360
Ala Gln Leu His Gln Ile Asn Ser Asn Asp Asp Arg Lys Leu Leu	365	370	375
Val Ser Tyr Leu Pro Thr Thr Val Ser Ala Lys Thr Asp Ile Ser	380	385	390
Ile Cys Ser Gly Leu Lys Lys Gly Phe Glu Val Val Glu Lys Leu	395	400	405
Asn Gly Lys Ala Tyr Gly Ser Val Met Ile Leu Val Thr Ser Gly	410	415	420
Asp Asp Lys Leu Leu Gly Asn Cys Leu Pro Thr Val Leu Ser Ser	425	430	435

Gly Ser Thr Ile His Ser Ile Ala Leu Gly Ser Ser Ala Ala Pro	440	445	450
Asn Leu Glu Glu Leu Ser Arg Leu Thr Gly Gly Leu Lys Phe Phe	455	460	465
Val Pro Asp Ile Ser Asn Ser Asn Ser Met Ile Asp Ala Phe Ser	470	475	480
Arg Ile Ser Ser Gly Thr Gly Asp Ile Phe Gln Gln His Ile Gln	485	490	495
Leu Glu Ser Thr Gly Glu Asn Val Lys Pro His His Gln Leu Lys	500	505	510
Asn Thr Val Thr Val Asp Asn Thr Val Gly Asn Asp Thr Met Phe	515	520	525
Leu Val Thr Trp Gln Ala Ser Gly Pro Pro Glu Ile Ile Leu Phe	530	535	540
Asp Pro Asp Gly Arg Lys Tyr Tyr Thr Asn Asn Phe Ile Thr Asn	545	550	555
Leu Thr Phe Arg Thr Ala Ser Leu Trp Ile Pro Gly Thr Ala Lys	560	565	570
Pro Gly His Trp Thr Tyr Thr Leu Asn Asn Thr His His Ser Leu	575	580	585
Gln Ala Leu Lys Val Thr Val Thr Ser Arg Ala Ser Asn Ser Ala	590	595	600
Val Pro Pro Ala Thr Val Glu Ala Phe Val Glu Arg Asp Ser Leu	605	610	615
His Phe Pro His Pro Val Met Ile Tyr Ala Asn Val Lys Gln Gly	620	625	630
Phe Tyr Pro Ile Leu Asn Ala Thr Val Thr Ala Thr Val Glu Pro	635	640	645
Glu Thr Gly Asp Pro Val Thr Leu Arg Leu Leu Asp Asp Gly Ala	650	655	660
Gly Ala Asp Val Ile Lys Asn Asp Gly Ile Tyr Ser Arg Tyr Phe	665	670	675
Phe Ser Phe Ala Ala Asn Gly Arg Tyr Ser Leu Lys Val His Val	680	685	690
Asn His Ser Pro Ser Ile Ser Thr Pro Ala His Ser Ile Pro Gly	695	700	705
Ser His Ala Met Tyr Val Pro Gly Tyr Thr Ala Asn Gly Asn Ile	710	715	720
Gln Met Asn Ala Pro Arg Lys Ser Val Gly Arg Asn Glu Glu Glu	725	730	735
Arg Lys Trp Gly Phe Ser Arg Val Ser Ser Gly Gly Ser Phe Ser	740	745	750
Val Leu Gly Val Pro Ala Gly Pro His Pro Asp Val Phe Pro Pro	755	760	765
Cys Lys Ile Ile Asp Leu Glu Ala Val Lys Val Glu Glu Glu Leu	770	775	780
Thr Leu Ser Trp Thr Ala Pro Gly Glu Asp Phe Asp Gln Gly Gln	785	790	795
Ala Thr Ser Tyr Glu Ile Arg Met Ser Lys Ser Leu Gln Asn Ile	800	805	810
Gln Asp Asp Phe Asn Asn Ala Ile Leu Val Asn Thr Ser Lys Arg	815	820	825
Asn Pro Gln Gln Ala Gly Ile Arg Glu Ile Phe Thr Phe Ser Pro	830	835	840
Gln Ile Ser Thr Asn Gly Pro Glu His Gln Pro Asn Gly Glu Thr	845	850	855



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Ile Gly Met Leu Asp	Ile Ala Leu Asn	Glu Ala Phe Asn	Ile Leu
245		250	255
Ser Asp Phe Asn His Thr Gly Gln Gly	Ser Ile Cys Ser Gln	Ala	
260		265	270
Ile Met Leu Ile Thr Asp Gly Ala Val	Asp Thr Tyr Asp Thr	Ile	
275		280	285
Phe Ala Lys Tyr Asn Trp Pro Asp Arg	Lys Val Arg Ile Phe	Thr	
290		295	300
Tyr Leu Ile Gly Arg Glu Ala Ala Phe	Ala Asp Asn Leu Lys	Trp	
305		310	315
Met Ala Cys Ala Asn Lys Gly Phe Phe	Thr Gln Ile Ser Thr	Leu	
320		325	330
Ala Asp Val Gln Glu Asn Val Met Glu Tyr	Leu His Val Leu	Ser	
335		340	345
Arg Pro Lys Val Ile Asp Gln Glu His	Asp Val Val Trp Thr	Glu	
350		355	360
Ala Tyr Ile Asp Ser Thr Leu Pro Gln	Ala Gln Lys Leu Thr	Asp	
365		370	375
Asp Gln Gly Pro Val Leu Met Thr Thr	Val Ala Met Pro Val	Phe	
380		385	390
Ser Lys Gln Asn Glu Thr Arg Ser Lys	Gly Ile Leu Leu Gly	Val	
395		400	405
Val Gly Thr Asp Val Pro Val Lys Glu	Leu Leu Lys Thr Ile	Pro	
410		415	420
Lys Tyr Lys Leu Gly Ile His Gly Tyr	Ala Phe Ala Ile Thr	Asn	
425		430	435
Asn Gly Tyr Ile Leu Thr His Pro Glu	Leu Arg Leu Leu Tyr	Glu	
440		445	450
Glu Gly Lys Lys Arg Arg Lys Pro Asn	Tyr Ser Ser Val Asp	Leu	
455		460	465
Ser Glu Val Glu Trp Glu Asp Arg Asp	Asp Val Leu Arg Asn	Ala	
470		475	480
Met Val Asn Arg Lys Thr Gly Lys Phe	Ser Met Glu Val Lys	Lys	
485		490	495
Thr Val Asp Lys Gly Val His Phe Ser	Gln Thr Phe Leu Leu	Leu	
500		505	510
Asn Leu Lys Gln Thr Thr Val Lys Asn			
515			

<210> 11

<211> 251

<212> PRT

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 3342358CD1

<400> 11

Met Thr Asp Ser Ala Thr Ala Asn Gly Asp Asp Arg Asp Pro Glu
1 5 10 15
Ile Glu Leu Phe Val Lys Ala Gly Ile Asp Gly Glu Ser Ile Gly
20 25 30
Asn Cys Pro Phe Ser Gln Arg Leu Phe Met Ile Leu Trp Leu Lys
35 40 45

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Cys	Ile	Ala	Ala	Ser	Glu	Phe	Tyr	Lys	Thr	Arg	His	Asn	Ile	Ile
				125					130					135
Leu	Ser	Ala	Gly	Ile	Phe	Phe	Val	Ser	Ala	Gly	Leu	Ser	Asn	Ile
				140					145					150
Ile	Gly	Ile	Ile	Val	Tyr	Ile	Ser	Ala	Asn	Ala	Gly	Asp	Pro	Ser
				155					160					165
Lys	Ser	Asp	Ser	Lys	Lys	Asn	Ser	Tyr	Ser	Tyr	Gly	Trp	Ser	Phe
				170					175					180
Tyr	Phe	Gly	Ala	Leu	Ser	Phe	Ile	Ile	Ala	Glu	Met	Val	Gly	Val
				185					190					195
Leu	Ala	Val	His	Met	Phe	Ile	Asp	Arg	His	Lys	Gln	Leu	Arg	Ala
				200					205					210
Thr	Ala	Arg	Ala	Thr	Asp	Tyr	Leu	Gln	Ala	Ser	Ala	Ile	Thr	Arg
				215					220					225
Ile	Pro	Ser	Tyr	Arg	Tyr	Arg	Tyr	Gln	Arg	Arg	Ser	Arg	Ser	Ser
				230					235					240
Ser	Arg	Ser	Thr	Glu	Pro	Ser	His	Ser	Arg	Asp	Ala	Ser	Pro	Val
				245					250					255
Gly	Ile	Lys	Gly	Phe	Asn	Thr	Leu	Pro	Ser	Thr	Glu	Ile	Ser	Met
				260					265					270
Tyr	Thr	Leu	Ser	Arg	Asp	Pro	Leu	Lys	Ala	Ala	Thr	Thr	Pro	Thr
				275					280					285
Ala	Thr	Tyr	Asn	Ser	Asp	Arg	Asp	Asn	Ser	Phe	Leu	Gln	Val	His
				290					295					300
Asn	Cys	Ile	Gln	Lys	Glu	Asn	Lys	Asp	Ser	Leu	His	Ser	Asn	Thr
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Ala	Asn	Arg	Arg	Thr	Thr	Pro	Val							
				320										

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 <211> 51  
 <212> PRT  
 <213> Homo sapiens  
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 <223> Incyte ID No: 1817329CD1

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 Lys Gly Ala Arg Arg Lys Asn Arg Phe Lys Gly Ser Asp Gly Ser  
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 Thr Ser Ser Asp Thr Thr Ser Asn Ser Phe Val Arg Gln Val Arg  
 35 40 45  
 Val Leu Ser Ser Trp Phe  
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<210> 14  
 <211> 113  
 <212> PRT  
 <213> Homo sapiens  
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<221> misc\_feature

<223> Incyte ID No: 3273307CD1

<400> 14

Met	Glu	Gln	Arg	Lys	Leu	Asn	Asp	Gln	Ala	Asn	Thr	Leu	Val	Asp	
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Leu	Ala	Lys	Thr	Gln	Asn	Ile	Met	Tyr	Asp	Met	Ile	Ser	Asp	Leu	
				20					25					30	
Asn	Glu	Arg	Ser	Glu	Asp	Phe	Glu	Lys	Arg	Ile	Val	Thr	Leu	Glu	
				35					40					45	
Thr	Lys	Leu	Glu	Thr	Leu	Ile	Gly	Ser	Ile	His	Ala	Leu	Pro	Gly	
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Leu	Ile	Ser	Gln	Thr	Ile	Arg	Gln	Gln	Gln	Arg	Asp	Phe	Ile	Glu	
				65					70					75	
Ala	Gln	Met	Glu	Ser	Tyr	Asp	Lys	His	Val	Thr	Tyr	Asn	Ala	Glu	
				80					85					90	
Arg	Ser	Arg	Ser	Ser	Ser	Arg	Arg	Arg	Arg	Ser	Ser	Ser	Thr	Ala	
				95					100					105	
Pro	Pro	Thr	Ser	Ser	Glu	Ser	Ser								
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<210> 15

<211> 215

<212> PRT

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 3824833CD1

<400> 15

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Gly	Leu	Ser	Leu	Phe	Phe	Ser	Leu	Val	Pro	Pro	Gly	Arg	Ser	Met	
				20					25					30	
Glu	Val	Thr	Val	Pro	Ala	Thr	Leu	Asn	Val	Leu	Asn	Gly	Ser	Asp	
				35					40					45	
Ala	Arg	Leu	Pro	Cys	Thr	Phe	Asn	Ser	Cys	Tyr	Thr	Val	Asn	His	
				50					55					60	
Lys	Gln	Phe	Ser	Leu	Asn	Trp	Thr	Tyr	Gln	Glu	Cys	Asn	Asn	Cys	
				65					70					75	
Ser	Glu	Glu	Met	Phe	Leu	Gln	Phe	Arg	Met	Lys	Ile	Ile	Asn	Leu	
				80					85					90	
Lys	Leu	Glu	Arg	Phe	Gln	Asp	Arg	Val	Glu	Phe	Ser	Gly	Asn	Pro	
				95					100					105	
Ser	Lys	Tyr	Asp	Val	Ser	Val	Met	Leu	Arg	Asn	Val	Gln	Pro	Glu	
				110					115					120	
Asp	Glu	Gly	Ile	Tyr	Asn	Cys	Tyr	Ile	Met	Asn	Pro	Pro	Asp	Arg	
				125					130					135	
His	Arg	Gly	His	Gly	Lys	Ile	His	Leu	Gln	Val	Leu	Met	Glu	Glu	
				140					145					150	
Pro	Pro	Glu	Arg	Asp	Ser	Thr	Val	Ala	Val	Ile	Val	Gly	Ala	Ser	
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Val	Gly	Gly	Phe	Leu	Ala	Val	Val	Ile	Leu	Val	Leu	Met	Val	Val	
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 <223> Incyte ID No: 2243917CD1

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                   20                  25                  30  
 Gly Leu Gly Ala Leu Ala Gln Arg Pro Gly Ser Val Ser Lys Trp  
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 Val Arg Leu Asn Val Gly Gly Thr Tyr Phe Leu Thr Thr Arg Gln  
                   50                  55                  60  
 Thr Leu Cys Arg Asp Pro Lys Ser Phe Leu Tyr Arg Leu Cys Gln  
                   65                  70                  75  
 Ala Asp Pro Asp Leu Asp Ser Asp Lys Asp Glu Thr Gly Ala Tyr  
                   80                  85                  90  
 Leu Ile Asp Arg Asp Pro Thr Tyr Phe Gly Pro Val Leu Asn Tyr  
                   95                  100                 105  
 Leu Arg His Gly Lys Leu Val Ile Asn Lys Asp Leu Ala Glu Glu  
                  110                 115                 120  
 Gly Val Leu Glu Glu Ala Glu Phe Tyr Asn Ile Thr Ser Leu Ile  
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 Lys Leu Val Lys Asp Lys Ile Arg Glu Arg Asp Ser Lys Thr Ser  
                  140                 145                 150  
 Gln Val Pro Val Lys His Val Tyr Arg Val Leu Gln Cys Gln Glu  
                  155                 160                 165  
 Glu Glu Leu Thr Gln Met Val Ser Thr Met Ser Asp Gly Trp Lys  
                  170                 175                 180  
 Phe Glu Gln Leu Val Ser Ile Gly Ser Ser Tyr Asn Tyr Gly Asn  
                  185                 190                 195  
 Glu Asp Gln Ala Glu Phe Leu Cys Val Val Ser Lys Glu Leu His  
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 Asn Thr Pro Tyr Gly Thr Ala Ser Glu Pro Ser Glu Lys Ala Lys  
                  215                 220                 225  
 Ile Leu Gln Glu Arg Gly Ser Arg Met  
                  230

<210> 18  
 <211> 301  
 <212> PRT  
 <213> Homo sapiens

<220>  
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 <223> Incyte ID No: 2597476CD1

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                   20                  25                  30  
 Val Phe Val Leu Met Leu Leu Thr Gln Gly Ala Val Ala Gln Ala  
                   35                  40                  45  
 Val Thr Ser Gly Glu Thr Lys Gly Asn Phe Phe Thr Met Phe Leu

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Ala Gly Ser Leu	Ala Val Thr Ile Ala Ile Tyr Val Gly Gly Asn	
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Val Ser Gly Ala	His Leu Asn Pro Ala Phe Ser Leu Ala Met Cys	
80	85	90
Ile Val Gly Arg	Leu Pro Trp Val Lys Leu Pro Ile Tyr Ile Leu	
95	100	105
Val Gln Leu Leu	Ser Ala Phe Cys Ala Ser Gly Ala Thr Tyr Val	
110	115	120
Leu Tyr His Asp	Ala Leu Gln Asn Tyr Thr Gly Gly Asn Leu Thr	
125	130	135
Val Thr Gly Pro	Lys Glu Thr Ala Ser Ile Phe Ala Thr Tyr Pro	
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Ala Pro Tyr Leu	Ser Leu Asn Asn Gly Phe Leu Asp Gln Val Leu	
155	160	165
Gly Thr Gly Met	Leu Ile Val Gly Leu Leu Ala Ile Leu Asp Arg	
170	175	180
Arg Asn Lys Gly	Val Pro Ala Gly Leu Glu Pro Val Val Val Gly	
185	190	195
Met Leu Ile Leu	Ala Leu Gly Leu Ser Met Gly Ala Asn Cys Gly	
200	205	210
Ile Pro Leu Asn	Pro Ala Arg Asp Leu Gly Pro Arg Leu Phe Thr	
215	220	225
Tyr Val Ala Gly	Trp Gly Pro Glu Val Phe Ser Ala Gly Asn Gly	
230	235	240
Trp Trp Trp Val	Pro Val Val Ala Pro Leu Val Gly Ala Thr Val	
245	250	255
Gly Thr Ala Thr	Tyr Gln Leu Leu Val Ala Leu His His Pro Glu	
260	265	270
Gly Pro Glu Pro	Ala Gln Asp Leu Val Ser Ala Gln His Lys Ala	
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Ser Glu Leu Glu	Thr Pro Ala Ser Ala Gln Met Leu Glu Cys Lys	
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Leu

<210> 19

<211> 2994

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 1568324CB1

<400> 19

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<210> 20

<211> 1298

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 4094907CB1

<400> 20

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ctcacaaaat ccaatgcgcc tgtccacatt gatgtgggag gccacatgta caccagcagc 240
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<210> 21

<211> 1877

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 518158CB1

<400> 21

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<210> 22

<211> 2517

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 602926CB1

<400> 22

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WO 00/12711

PCT/US99/20468

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<211> 1154

<212> DNA

<213> Homo sapiens

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<223> Incyte ID No: 922119CB1

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<211> 1879

<212> DNA

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<220>

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<223> Incyte ID No: 2666782CB1

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WO 00/12711

PCT/US99/20468

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<211> 1537

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 2731369CB1

<400> 25

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WO 00/12711

PCT/US99/20468

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<223> a or g or c or t, unknown, or other

<220>  
<221> misc\_feature  
<223> Incyte ID No: 1375415CB1

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<212> DNA  
<213> Homo sapiens

<220>  
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<211> 1774

<212> DNA

<213> Homo sapiens

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<221> misc\_feature

<223> Incyte ID No: 3148427CB1

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WO 00/12711

PCT/US99/20468

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<211> 1505

<212> DNA

<213> Homo sapiens

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<223> Incyte ID No: 3342358CB1

<400> 29

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WO 00/12711

PCT/US99/20468

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<213> Homo sapiens

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<210> 31  
<211> 1971  
<212> DNA  
<213> Homo sapiens

<220>  
<221> misc\_feature  
<223> Incyte ID No: 1817329CB1

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gctgaagatc tcatatggaa aaggagccag aaggaaaaac agatttaaag gatctgatgg 180
aagcacgtca tctgatacta cctcaaatag ttttgttcgc caggtaagag ttttaagttc 240
atggttttga taagtacctt aaaatgactt tagattttta aaggtgggtt tcaactcttt 300
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WO 00/12711

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ctggcaagtt gaggaagag aagtgtggca ttcattacag aggatttctt tgaaacagtg 480
agagaactcc agcagaaatg attatggatt tggggggatt gttttttttt ttttttttca 540
gtgggacaga gagctgatgt gcatctgtat ccctacctgt gagatactgg ggttcttcta 600
gttgagcttt cttcttctca cttgggcttg cattgaaaac tagaaaatca ttcttggtct 660
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<211> 1424

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 3273307CB1

<400> 32

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gtagtgcaca gccagaaata ccaagcaggc ttgcaaagga caagattaac cttggcctca 240
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tgaaggagta agatagaatt taccacagaa agaaagtggg agtatctccc tatttcaatt 420
taagtgttgt tcccaggcag gtacaagtac ttctaaaaag gcatagatta aagaaaaatg 480
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gttctgatgt cagaatcctg ggaacctgaa cactaagttt taggccaaaa tgagtgaata 1260
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<210> 33

<211> 1224

<212> DNA

<213> Homo sapiens

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<221> misc\_feature

<223> Incyte ID No: 3824833CB1

<400> 33

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tcacctctc ccgtagccca cccgactaac atctcagtct ctgaaaatgc acagagatgc 180
ctggctacct cgcctgcct tcagcctcac ggggctcagt ctctttttct ctttgggtgcc 240
accaggacgg agcatggagg tcacagtacc tgccaccctc aacgtcctca atggctctga 300
cgcccgctg ccttgacact tcaactcctg ctacacagtg aaccacaaac agttctccct 360
gaactggact taccaggagt gcaacaactg ctctgaggag atgttcctcc agttccgcat 420
gaagatcatt aacctgaagc tggagcggtt tcaagaccgc gtggagttct cagggaaccc 480
cagcaagtac gatgtgtcgg tgatgctgag aaacgtgcag ccggaggatg aggggattta 540
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<210> 34

<211> 1300

<212> DNA

<213> Homo sapiens

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<221> misc\_feature

<223> Incyte ID No: 2069907CB1

<400> 34

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aagtatgcag tacgctcttg ggtcgtctca tgagaccag gggcatgttg gaaagaactg 180
agagaaagag caacaaagcg gcgagtgtg tgagagggca gcacgcgctg tggggccctt 240
ccagagaaat gtactgaaaa agtctacgca atgtctggga tttgctaaac aatacctgga 300
aagcagacag gtctttttgc cattcctcca ggacatccac cataaggaaa ggagaccctg 360
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gaccaacatt ctctaagatg tttatatgga ccagtggccg gacctcttca tcttatagac 420
atgatgaaaa aagaaatatt taccagaaaa tcagggacca tgacctcctg gacaaaagga 480
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<210> 35

<211> 1060

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<223> Incyte ID No: 2243917CB1

<400> 35

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<210> 36

<211> 1815

<212> DNA

<213> Homo sapiens

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<400> 36

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1815

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<210> 37  
 <211> 315  
 <212> PRT  
 <213> Rattus norvegicus

<220>  
 <221> misc\_feature  
 <223> GenBank ID No: g2924369

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<400> 37
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20 25 30
Ser Lys Pro Glu Ala Asn Pro Gln Pro Glu Pro Glu Val Gln Pro
35 40 45
Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Ala
50 55 60
Pro Glu Glu Ala Ala Pro Glu Val Gln Thr Leu Pro Pro Glu Glu
65 70 75
Pro Val Glu Gly Glu Asp Val Ala Glu Ala Gly Pro Ser Leu Gln
80 85 90
Glu Thr Gln Glu Ala Asp Pro Pro Gln Pro Thr Ser Gln Ala Gln
95 100 105

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Trp	Phe	Trp	Lys	Gly	Met	Glu	Lys	Val	Val	Pro	Gln	Pro	Val	Tyr
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Ser	Ser	Ser	Gly	Gly	Gln	Asn	Leu	Ala	Ala	Gly	Glu	Gly	Gly	Pro
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Asp	Gln	Asp	Gly	Ala	Gln	Thr	Leu	Glu	Pro	Cys	Gly	Thr	Gly	Asp
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Pro	Gly	Ser	Glu	Asp	Gly	Ser	Asp	Lys	Thr	Ser	Lys	Thr	Gln	Asp
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Thr	Glu	Pro	Ser	Leu	Trp	Leu	Leu	Arg	Trp	Leu	Glu	Leu	Asn	Leu
				185					190					195
Glu	Lys	Val	Leu	Pro	Gln	Pro	Pro	Thr	Pro	Ser	Gln	Ala	Trp	Lys
				200					205					210
Val	Glu	Pro	Glu	Gly	Ala	Val	Leu	Glu	Pro	Asp	Pro	Pro	Gly	Thr
				215					220					225
Pro	Met	Glu	Val	Glu	Pro	Thr	Glu	Asn	Pro	Ser	Gln	Pro	Asn	Pro
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Gly	Pro	Val	Glu	Pro	Glu	Glu	Glu	Pro	Ala	Ala	Glu	Pro	Gln	Pro
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Gly	Phe	Gln	Ala	Ser	Ser	Leu	Pro	Pro	Pro	Gly	Asp	Pro	Val	Arg
				260					265					270
Leu	Ile	Glu	Trp	Leu	Leu	His	Arg	Leu	Glu	Met	Ala	Leu	Pro	Gln
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Pro	Val	Leu	His	Gly	Lys	Ala	Ala	Glu	Gln	Glu	Pro	Ser	Cys	Pro
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<210> 38  
 <211> 490  
 <212> PRT  
 <213> *Drosophila melanogaster*

<220>  
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 <223> GenBank ID No: g116443

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Lys	Tyr	Pro	Asp	Thr	Leu	Leu	Gly	Ser	Asn	Glu	Arg	Glu	Phe	Phe
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Tyr	Asp	Glu	Asp	Cys	Lys	Glu	Tyr	Phe	Phe	Asp	Arg	Asp	Pro	Asp
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Ile	Phe	Arg	His	Ile	Leu	Asn	Tyr	Tyr	Arg	Thr	Gly	Lys	Leu	His
				95					100					105
Tyr	Pro	Lys	His	Glu	Cys	Leu	Thr	Ser	Tyr	Asp	Glu	Glu	Leu	Ala
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Phe	Phe	Gly	Ile	Met	Pro	Asp	Val	Ile	Gly	Asp	Cys	Cys	Tyr	Glu
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Asp	Lys	Leu	Ser	Glu	Asn	Gly	Asp	Gln	Asn	Leu	Gln	Gln	Leu	Thr
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Asn	Met	Arg	Gln	Lys	Met	Trp	Arg	Ala	Phe	Glu	Asn	Pro	His	Thr
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Ser	Thr	Ser	Ala	Leu	Val	Phe	Tyr	Tyr	Val	Thr	Gly	Phe	Phe	Ile
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Ala	Val	Ser	Val	Met	Ala	Asn	Val	Val	Glu	Thr	Val	Pro	Cys	Gly
				200					205					210
His	Arg	Pro	Gly	Arg	Ala	Gly	Thr	Leu	Pro	Cys	Gly	Glu	Arg	Tyr
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Lys	Ile	Val	Phe	Phe	Cys	Leu	Asp	Thr	Ala	Cys	Val	Met	Ile	Phe
				230					235					240
Thr	Ala	Glu	Tyr	Leu	Leu	Arg	Leu	Phe	Ala	Ala	Pro	Asp	Arg	Cys
				245					250					255
Lys	Phe	Val	Arg	Ser	Val	Met	Ser	Ile	Ile	Asp	Val	Val	Ala	Ile
				260					265					270
Met	Pro	Tyr	Tyr	Ile	Gly	Leu	Gly	Ile	Thr	Asp	Asn	Asp	Asp	Val
				275					280					285
Ser	Gly	Ala	Phe	Val	Thr	Leu	Arg	Val	Phe	Arg	Val	Phe	Arg	Ile
				290					295					300
Phe	Lys	Phe	Ser	Arg	His	Ser	Gln	Gly	Leu	Arg	Ile	Leu	Gly	Tyr
				305					310					315
Thr	Leu	Lys	Ser	Cys	Ala	Ser	Glu	Leu	Gly	Phe	Leu	Val	Phe	Ser
				320					325					330
Leu	Ala	Met	Ala	Ile	Ile	Ile	Phe	Ala	Thr	Val	Met	Phe	Tyr	Ala
				335					340					345
Glu	Lys	Asn	Val	Asn	Gly	Thr	Asn	Phe	Thr	Ser	Ile	Pro	Ala	Ala
				350					355					360
Phe	Trp	Tyr	Thr	Ile	Val	Thr	Met	Thr	Thr	Leu	Gly	Tyr	Gly	Asp
				365					370					375
Met	Val	Pro	Glu	Thr	Ile	Ala	Gly	Lys	Ile	Val	Gly	Gly	Val	Cys
				380					385					390
Ser	Leu	Ser	Gly	Val	Leu	Val	Ile	Ala	Leu	Pro	Val	Pro	Val	Ile
				395					400					405
Val	Ser	Asn	Phe	Ser	Arg	Ile	Tyr	His	Gln	Asn	Gln	Arg	Ala	Asp
				410					415					420
Lys	Arg	Lys	Ala	Gln	Arg	Lys	Ala	Arg	Leu	Ala	Arg	Ile	Arg	Ile
				425					430					435
Ala	Lys	Ala	Ser	Ser	Gly	Ala	Ala	Phe	Val	Ser	Lys	Lys	Lys	Ala
				440					445					450
Ala	Glu	Ala	Arg	Trp	Ala	Ala	Gln	Glu	Ser	Gly	Ile	Glu	Leu	Asp
				455					460					465
Asp	Asn	Tyr	Arg	Asp	Glu	Asp	Ile	Phe	Glu	Leu	Gln	His	His	His
				470					475					480
Leu	Leu	Arg	Cys	Leu	Glu	Lys	Thr	Thr	Met					
				485					490					

<210> 39

<211> 478

<212> PRT

<213> Polyorchis penicillatus

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<220>

<221> misc\_feature

<223> GenBank ID No: g1763619

<400> 39

Met	Asn	Gly	Asp	Ile	Gly	Ala	Trp	Ile	Ser	Cys	Ala	Arg	Thr	Ala
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Gly	Ile	Gly	Trp	Val	Pro	Ile	Ser	Ser	Lys	Glu	Pro	Ser	Ala	Tyr
				20					25					30
Leu	Asn	Lys	Gln	Val	Cys	Asn	Glu	Asn	Glu	Lys	Asn	Asn	Ala	Lys
				35					40					45
Leu	Thr	Ile	Asn	Val	Ser	Gly	Arg	Arg	Tyr	Gln	Thr	Tyr	Ser	His
				50					55					60
Thr	Leu	Arg	Lys	Phe	Lys	Glu	Thr	Leu	Leu	Gly	Ser	Gln	Glu	Arg
				65					70					75
Asp	Tyr	Phe	Tyr	Asp	Glu	Ser	Leu	Glu	Glu	Tyr	Tyr	Phe	Asp	Arg
				80					85					90
Asp	Pro	Asp	Leu	Phe	Arg	His	Ile	Leu	Asn	Tyr	Tyr	Arg	Thr	Gly
				95					100					105
Lys	Leu	His	Phe	Pro	Lys	Asn	Glu	Cys	Val	Ser	Ser	Phe	Glu	Asp
				110					115					120
Glu	Leu	Thr	Phe	Phe	Gly	Ile	Lys	Gly	Phe	Asn	Ile	Asn	Asn	Cys
				125					130					135
Cys	Trp	Asp	Asp	Tyr	His	Asp	Lys	Lys	Arg	Glu	Cys	Thr	Glu	Arg
				140					145					150
Leu	Asn	Glu	Ser	Asp	Val	Met	Leu	Thr	Ser	Ser	Glu	Ile	Asn	Glu
				155					160					165
Lys	Ser	Asp	Thr	Met	Gly	Ile	Asp	Val	Gln	Met	Asn	Asn	His	Gln
				170					175					180
Ala	Lys	Asn	Phe	Arg	Gln	Lys	Val	His	Gly	Leu	Phe	Glu	Asn	Pro
				185					190					195
Gln	Ser	Thr	Phe	Leu	Ala	Arg	Ile	Leu	Tyr	Tyr	Ile	Thr	Gly	Phe
				200					205					210
Phe	Ile	Ala	Val	Ser	Val	Gly	Ser	Thr	Ile	Ile	Glu	Thr	Ile	Asp
				215					220					225
Cys	Ser	Ala	Asn	Arg	Pro	Cys	Gly	Glu	Val	Tyr	Asn	Lys	Ile	Phe
				230					235					240
Phe	Asn	Ile	Glu	Ala	Val	Cys	Val	Val	Val	Phe	Thr	Ile	Glu	Tyr
				245					250					255
Leu	Ala	Arg	Leu	Tyr	Ser	Ala	Pro	Cys	Arg	Phe	Arg	His	Ala	Arg
				260					265					270
Ile	Ser	Leu	Ser	Ile	Ile	Asp	Val	Ile	Ala	Ile	Leu	Pro	Phe	Tyr
				275					280					285
Ile	Gly	Leu	Ala	Met	Thr	Lys	Thr	Ser	Ile	Ser	Gly	Ala	Phe	Val
				290					295					300
Ser	Leu	Arg	Val	Phe	Arg	Val	Phe	Arg	Ile	Phe	Lys	Phe	Ser	Arg
				305					310					315
His	Ser	Lys	Gly	Leu	Arg	Ile	Leu	Gly	Ser	Thr	Leu	Thr	Ser	Cys
				320					325					330
Ala	Ser	Glu	Leu	Gly	Phe	Leu	Leu	Phe	Ser	Leu	Ser	Met	Ala	Ile
				335					340					345
Ile	Ile	Phe	Ala	Thr	Val	Val	Phe	Tyr	Val	Glu	Lys	Asp	Val	Asn
				350					355					360
Asp	Ser	Asp	Phe	Thr	Ser	Ile	Pro	Ala	Ser	Phe	Trp	Tyr	Thr	Ile
				365					370					375
Val	Thr	Met	Thr	Thr	Leu	Gly	Tyr	Gly	Asp	Met	Val	Pro	Lys	Thr

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Ile	Pro	Gly	Lys	Leu	Val	Gly	Ser	Ile	Cys	Ser	Leu	Ser	Gly	Val
				380					385					390
Leu	Val	Ile	Ala	Leu	Pro	Val	Pro	Val	Ile	Val	Ser	Asn	Phe	Ser
				395					400					405
Arg	Ile	Tyr	Leu	Gln	Asn	Gln	Arg	Ala	Asp	Lys	Arg	Arg	Ala	Asn
				410					415					420
Gln	Lys	Leu	Arg	Asn	Lys	Cys	Glu	Glu	Lys	Glu	Glu	Lys	Lys	Lys
				425					430					435
Glu	Ser	Ser	Ser	Glu	Thr	Val	Thr	Arg	Phe	Ile	Ile	Ser	Asn	Gln
				440					445					450
Met	Tyr	Thr	Ile	Phe	Ser	Met	Lys	Phe	Ala	Leu	Thr	Arg		
				455					460					465
				470					475					

<210> 40  
 <211> 732  
 <212> PRT  
 <213> Rattus norvegicus

<220>  
 <221> misc\_feature  
 <223> GenBank ID No: g2564072

<400> 40

Met	Asp	Thr	Ser	Gly	His	Phe	His	Glu	Ser	Gly	Val	Gly	Asp	Leu
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Asp	Glu	Asp	Pro	Lys	Cys	Pro	Cys	Pro	Ser	Ser	Gly	Asp	Glu	Gln
				20				25					30	
Gln	Gln	Gln	Gln	Gln	Pro	Pro	Pro	Pro	Ser	Ala	Pro	Pro	Ala	Val
				35				40					45	
Pro	Gln	Gln	Pro	Pro	Gly	Pro	Leu	Leu	Gln	Pro	Gln	Pro	Pro	Gln
				50				55					60	
Leu	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln
				65				70					75	
Gln	Gln	Gln	Gln	Gln	Ala	Pro	Leu	His	Pro	Leu	Pro	Gln	Leu	Ala
				80				85					90	
Gln	Leu	Gln	Ser	Gln	Val	Val	His	Pro	Gly	Leu	Leu	His	Ser	Ser
				95				100					105	
Pro	Thr	Ala	Phe	Arg	Ala	Pro	Asn	Ser	Ala	Asn	Ser	Thr	Ala	Ile
				110				115					120	
Leu	His	Pro	Ser	Ser	Arg	Gln	Gly	Ser	Gln	Leu	Asn	Leu	Asn	Asp
				125				130					135	
His	Leu	Val	Gly	His	Ser	Pro	Ser	Ser	Thr	Ala	Thr	Ser	Gly	Pro
				140				145					150	
Gly	Gly	Gly	Ser	Arg	His	Arg	Gln	Ala	Ser	Pro	Val	Val	His	Arg
				155				160					165	
Arg	Asp	Ser	Asn	Pro	Phe	Thr	Glu	Ile	Ala	Met	Ser	Ser	Cys	Lys
				170				175					180	
Tyr	Ser	Gly	Gly	Val	Met	Lys	Pro	Leu	Asn	Arg	Leu	Ser	Ala	Ser
				185				190					195	
Arg	Arg	Asn	Leu	Ile	Glu	Ala	Glu	Pro	Glu	Gly	Gln	Pro	Leu	Gln
				200				205					210	
Leu	Phe	Ser	Pro	Ser	Asn	Pro	Pro	Glu	Ile	Ile	Ile	Ser	Ser	Arg
				215				220					225	
Glu	Asp	Asn	His	Ala	His	Gln	Thr	Leu	Leu	His	His	Pro	Asn	Ala

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	230		235		240
Thr His Asn His	Gln His Ala Gly Thr	Thr Ala Gly Ser Thr Thr			
	245		250		255
Phe Pro Lys Ala	Asn Lys Arg Lys Asn	Gln Asn Ile Gly Tyr Lys			
	260		265		270
Leu Gly His Arg	Arg Ala Leu Phe Glu	Lys Arg Lys Arg Leu Ser			
	275		280		285
Asp Tyr Ala Leu	Ile Phe Gly Met Phe	Gly Ile Val Val Met Val			
	290		295		300
Ile Glu Thr Glu	Leu Ser Trp Gly Leu	Tyr Ser Lys Asp Ser Met			
	305		310		315
Phe Ser Leu Ala	Leu Lys Cys Leu Ile	Ser Leu Ser Thr Ile Ile			
	320		325		330
Leu Leu Gly Leu	Ile Ile Ala Tyr His	Thr Arg Glu Val Gln Leu			
	335		340		345
Phe Val Ile Asp	Asn Gly Ala Asp Asp	Trp Arg Ile Ala Met Thr			
	350		355		360
Tyr Glu Arg Ile	Leu Tyr Ile Ser Leu	Glu Met Leu Val Cys Ala			
	365		370		375
Ile His Pro Ile	Pro Gly Glu Tyr Lys	Phe Phe Trp Thr Ala Arg			
	380		385		390
Leu Ala Phe Ser	Tyr Thr Pro Ser Arg	Ala Glu Ala Asp Val Asp			
	395		400		405
Ile Ile Leu Ser	Ile Pro Met Phe Leu	Arg Leu Tyr Leu Ile Ala			
	410		415		420
Arg Val Met Leu	Leu His Ser Lys Leu	Phe Thr Asp Ala Ser Ser			
	425		430		435
Arg Ser Ile Gly	Ala Leu Asn Lys Ile	Asn Phe Asn Thr Arg Phe			
	440		445		450
Val Met Lys Thr	Leu Met Thr Ile Cys	Pro Gly Thr Val Leu Leu			
	455		460		465
Val Phe Ser Ile	Ser Leu Trp Ile Ile	Ala Ala Trp Thr Val Arg			
	470		475		480
Val Cys Glu Arg	Tyr His Asp Gln Gln	Asp Val Thr Ser Asn Phe			
	485		490		495
Leu Gly Ala Met	Trp Leu Ile Ser Ile	Thr Phe Leu Ser Ile Gly			
	500		505		510
Tyr Gly Asp Met	Val Pro His Thr Tyr	Cys Gly Lys Gly Val Cys			
	515		520		525
Leu Leu Thr Gly	Ile Met Gly Ala Gly	Cys Thr Ala Leu Val Val			
	530		535		540
Ala Val Val Ala	Arg Lys Leu Glu Leu	Thr Lys Ala Glu Lys His			
	545		550		555
Val His Asn Phe	Met Met Asp Thr Gln	Leu Thr Lys Arg Ile Lys			
	560		565		570
Asn Ala Ala Ala	Asn Val Leu Arg Glu	Thr Trp Leu Ile Tyr Lys			
	575		580		585
His Thr Lys Leu	Leu Lys Lys Ile Asp	His Ala Lys Val Arg Lys			
	590		595		600
His Gln Arg Lys	Phe Leu Gln Ala Ile	His Gln Leu Arg Gly Val			
	605		610		615
Lys Met Glu Gln	Arg Lys Leu Ser Asp	Gln Ala Asn Thr Leu Val			
	620		625		630
Asp Leu Ser Lys	Met Gln Asn Val Met	Tyr Asp Leu Ile Thr Glu			
	635		640		645
Leu Asn Asp Arg	Ser Glu Asp Leu Glu	Lys Gln Ile Gly Ser Leu			

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	650		655		660
Glu Ser Lys Leu	Glu His Leu Thr Ala	Ser Phe Asn Ser Leu	Pro		
	665		670		675
Leu Leu Ile Ala	Asp Thr Leu Arg Gln	Gln Gln Gln Gln Leu	Leu		
	680		685		690
Thr Ala Phe Val	Glu Ala Arg Gly Ile	Ser Val Ala Val Gly	Thr		
	695		700		705
Ser His Ala Pro	Pro Ser Asp Ser Pro	Ile Gly Ile Ser Ser	Thr		
	710		715		720
Ser Phe Pro Thr	Pro Tyr Thr Ser Ser	Ser Ser Cys			
	725		730		

<210> 41  
 <211> 269  
 <212> PRT  
 <213> Rattus norvegicus

<220>  
 <221> misc\_feature  
 <223> GenBank ID No: g2350843

<400> 41

Met Ala Gly Ser Val	Leu Glu Asn Ile	Gln Ser Val Leu	Gln Lys
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Thr Trp Val Arg	Glu Phe Leu Ala	Glu Phe Leu Asn Thr	Tyr Val
	20	25	30
Leu Met Val Phe	Gly Leu Gly Ser Val	Ala His Met Val Leu	Gly
	35	40	45
Glu Arg Leu Gly	Ser Tyr Leu Gly Val	Asn Leu Gly Phe Gly	Phe
	50	55	60
Gly Val Thr Met	Gly Ile His Val Ala	Gly Gly Ile Ser Gly	Ala
	65	70	75
His Met Asn Pro	Ala Val Thr Phe Thr	Asn Cys Ala Leu Gly	Arg
	80	85	90
Met Ala Gly Arg	Lys Phe Pro Ile Tyr	Val Leu Gly Gln Phe	Leu
	95	100	105
Gly Ser Phe Leu	Ala Ala Thr Thr	Tyr Leu Ile Phe Tyr	Gly
	110	115	120
Ala Ile Asn His	Tyr Ala Gly Glu Thr	Leu Leu Val Thr Gly	Pro
	125	130	135
Lys Ser Thr Ala	Asn Ile Phe Ala Thr	Tyr Leu Pro Glu His	Met
	140	145	150
Thr Leu Trp Arg	Gly Phe Val Asp Glu	Val Phe Val Thr Gly	Met
	155	160	165
Leu Gln Leu Cys	Ile Phe Ala Ile Thr	Asp Lys Leu Asn Ser	Pro
	170	175	180
Ala Leu Gln Gly	Thr Glu Pro Leu Met	Ile Gly Ile Leu Val	Cys
	185	190	195
Val Leu Gly Val	Ser Leu Gly Met Asn	Thr Gly Tyr Ala Ile	Asn
	200	205	210
Pro Ser Arg Asp	Leu Pro Pro Arg Phe	Phe Thr Phe Ile Ala	Gly
	215	220	225
Trp Gly Lys Lys	Val Phe Ser Ala Gly	Asn Asn Trp Trp Trp	Val
	230	235	240
Pro Val Val Ala	Pro Leu Leu Gly Ala	Tyr Leu Gly Gly Ile	Val



Tyr Leu Gly Leu Ile His Ala Gly Ile Pro Pro Gln Gly Ser  
 245 250 255  
 260 265

<210> 42  
 <211> 266  
 <212> PRT  
 <213> Mus musculus

<220>  
 <221> misc\_feature  
 <223> GenBank ID No: g192647

<400> 42  
 Met Asn Trp Gly Phe Leu Gln Gly Ile Leu Ser Gly Val Asn Lys  
 1 5 10 15  
 Tyr Ser Thr Ala Leu Gly Arg Ile Trp Leu Ser Val Val Phe Ile  
 20 25 30  
 Phe Arg Val Leu Val Tyr Val Val Ala Ala Glu Glu Val Trp Asp  
 35 40 45  
 Asp Asp Gln Lys Asp Phe Ile Cys Asn Thr Lys Gln Pro Gly Cys  
 50 55 60  
 Pro Asn Val Cys Tyr Asp Glu Phe Phe Pro Val Ser His Val Arg  
 65 70 75  
 Leu Trp Ala Leu Gln Leu Ile Leu Val Thr Cys Pro Ser Leu Leu  
 80 85 90  
 Val Val Met His Val Ala Tyr Arg Glu Glu Arg Glu Arg Lys His  
 95 100 105  
 Arg Leu Lys His Gly Pro Asn Ala Pro Ala Leu Tyr Ser Asn Leu  
 110 115 120  
 Ser Lys Lys Arg Gly Gly Leu Trp Trp Thr Tyr Leu Leu Ser Leu  
 125 130 135  
 Ile Phe Lys Ala Ala Val Asp Ser Gly Phe Leu Tyr Ile Phe His  
 140 145 150  
 Cys Ile Tyr Lys Asp Tyr Asp Met Pro Arg Val Val Ala Cys Ser  
 155 160 165  
 Val Thr Pro Cys Pro His Thr Val Asp Cys Tyr Ile Ala Arg Pro  
 170 175 180  
 Thr Glu Lys Lys Val Phe Thr Tyr Phe Met Val Val Thr Ala Ala  
 185 190 195  
 Ile Cys Ile Leu Leu Asn Leu Ser Glu Val Val Tyr Leu Val Gly  
 200 205 210  
 Lys Arg Cys Met Glu Val Phe Arg Pro Arg Arg Arg Lys Ala Ser  
 215 220 225  
 Arg Arg His Gln Leu Pro Asp Thr Cys Pro Pro Tyr Val Ile Ser  
 230 235 240  
 Lys Gly Gly His Pro Gln Asp Glu Ser Val Ile Leu Thr Lys Ala  
 245 250 255  
 Gly Met Ala Thr Val Asp Ala Gly Val Tyr Pro  
 260 265

<210> 43  
 <211> 191  
 <212> PRT



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      80      85      90
Leu Asp Gly Val Ser Glu Glu Gly Val Leu Glu Glu Ala Glu Phe
      95      100      105
Tyr Asn Val Thr Gln Leu Ile Ala Leu Leu Lys Glu Cys Ile Leu
      110      115      120
His Arg Asp Gln Arg Pro Gln Thr Asp Lys Lys Arg Val Tyr Arg
      125      130      135
Val Leu Gln Cys Arg Glu Gln Glu Leu Thr Gln Met Ile Ser Thr
      140      145      150
Leu Ser Asp Gly Trp Arg Phe Glu Gln Leu Ile Ser Met Gln Tyr
      155      160      165
Thr Asn Tyr Gly Pro Phe Glu Asn Asn Glu Phe Leu Cys Val Val
      170      175      180
Ser Lys Glu Cys Gly Thr Thr Ala Gly Arg Glu Leu Glu Leu Asn
      185      190      195
Asp Arg Ala Lys Val Leu Gln Gln Lys Gly Ser Arg Ile Asn Thr
      200      205      210
Ile Ser His Ser Ala Thr Pro Thr Gln His Gln Leu Asp Ala Ala
      215      220      225
Lys Glu Ala Arg Ala Thr Ala Thr Ala Thr Ser Asn Thr Thr Asn
      230      235      240
His Thr Arg Ser Asp Gln Thr Gln Pro Gln Ala Gln Ile Thr His
      245      250      255
Gln Asp Gln Pro Glu Ser Pro Lys Gln Ser Pro Gln Gly Asp Tyr
      260      265      270
Ala Ser Phe Ala Phe Glu Thr Lys Leu Thr Gly Thr Thr Ala Ile
      275      280      285
Arg Phe Ser Pro Leu Trp Pro Phe Cys Ala Leu Tyr Glu Val Cys
      290      295      300
Ala Gly Val His Val Phe Asn Leu
      305

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&lt;210&gt; 45

&lt;211&gt; 295

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; GenBank ID No: g2887407

&lt;400&gt; 45

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Met Gln Pro Glu Gly Ala Glu Lys Gly Lys Ser Phe Lys Gln Arg
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Leu Val Leu Lys Ser Ser Leu Ala Lys Glu Thr Leu Ser Glu Phe
      20      25      30
Leu Gly Thr Phe Ile Leu Ile Val Leu Gly Cys Gly Cys Val Ala
      35      40      45
Gln Ala Ile Leu Ser Arg Gly Arg Phe Gly Gly Val Ile Thr Ile
      50      55      60
Asn Val Gly Phe Ser Met Ala Val Ala Met Ala Ile Tyr Val Ala
      65      70      75
Gly Gly Val Ser Gly Gly His Ile Asn Pro Ala Val Ser Leu Ala
      80      85      90
Met Cys Leu Phe Gly Arg Met Lys Trp Phe Lys Leu Pro Phe Tyr

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